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CORRIGENDA IN VOL. XXIII

p. 307, line 10: *for* "$(4/5)^1$" *read* "$(4/5)^3$"
   ,,   ,, 19: *for* "$(5/6)^2$" *read* "$(5/6)^4$"

THE INHERITANCE OF THE CAPACITY FOR SHOWING MUTUAL AVERSION BETWEEN MONO-SPORE MYCELIA OF *DIAPORTHE PERNICIOSA* (Marchal).

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(With Plate I, Three Schemes, Nine Charts and Four Tables.)

CONTENTS

INTRODUCTION.

Aversion in Diaporthe perniciosa.

A PRELIMINARY paper on the phenomenon of mutual aversion between monospore mycelia of the Ascomycetous fungus *Diaporthe perniciosa* (March.) was published in 1923. Since that date, further and more ex-

tensive work has been done on the investigation of the inheritance of this capacity for showing aversion.

In the preliminary paper (Cayley, 1923 *b*) two forms of *Diaporthe* associated with "die-back" of fruit trees were described. The one was haplo-synoecious as regards sex[1], and produced perithecia freely in mono-spore cultures, but the pycnidia contained only one kind of spore, namely, the oval "a" spore. No filiform "b" spores were found in the pycnidia of this form. Mycelia from the same perithecium never showed aversion *inter se*, although mycelia from different sources were capable of showing aversion towards one another. Moreover, the progeny from monospore cultures remained true to the parent type, inherited the capacity for showing aversion, and showed the same reactions as the parent towards all mycelia with which they were tested.

The second form has been found to be haplo-heteroecious. The pycnidia contain both oval "a" and filiform "b" spores, and monospore mycelia not only show aversion between mycelia from different sources but are also capable of showing aversion towards other mycelia from the same perithecium.

It is now thought that the two forms are probably closely allied but distinct species. They both have, and inherit, the capacity for showing *inter*-racial aversion, but *intra*-perithecial aversion has only been found in the second form, in which "b" pycnospores are invariably present.

The present paper deals entirely with this second form.

Aversion in *Diaporthe* is the outward and visible sign of a physiological heterothallism, which is not a case of simple haplo-heteroecism. The results obtained from a large number of aversion tests, comprising some 1300 mono-ascus and mono-ascospore isolations derived from a single pycnospore culture, has proved that the capacity for showing aversion is a heritable character, and segregates in a very definite manner in the second and subsequent ascospore generations. The more recent results have also shown *D. perniciosa* to be haplo-heteroecious as well as heterothallic as regards aversion, but these two forms of heterothallism do not appear to be closely correlated.

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[1] In view of the different forms of heterothallism to be described in this paper the terminology suggested by Correns (1928) and employed by Hartmann (1929) for sex-heterothallism in the Protista and Thallophyta has been adopted as far as *Diaporthe* is concerned, as follows:

Haplo-synoecious = homothallic.	Diplo-synoecious = homophytic.
Haplo-heteroecious = heterothallic.	Diplo-heteroecious = heterophytic.

The term heterothallism is used in its wider sense to denote both morphological and physiological differences other than sex.

A statement made in the previous paper on aversion (Cayley, 1923 *b*) to the effect that "the averting strains on the same host may be due to multiple infection from two or more different sources, and not to the splitting up into physiological strains in the host plant," must now be modified. Multiple infection of course is not improbable, but the presence of averting strains in the same host must be mainly due to the segregation of the factor or factors for aversion carried by the mycelia.

No cytological evidence can be brought to bear on the problem. The perithecia and nuclei are so small, and technical difficulties such as fixation and penetration through the thick wall of the perithecium so great, that this side of the investigation has had to be abandoned.

The fact that the mycelia show aversion towards each other may mean that one mycelium produces or excretes a chemical substance (possibly volatile) and the other mycelium a complimentary substance, the meeting of which sets up some reaction which results in the death or much retarded growth of the hyphae along the line of contact. If two pieces of the same monospore mycelium are used as inocula in the same petri dish, the colonies resulting from these inocula invariably intermingle quite freely, although the same individual may be capable of showing aversion to other mono-mycelia from different sources, and even, in some instances, to mycelia derived from the same perithecium. This shows that aversion is not due to staling of the medium, used in the strict sense, although staling may be set up by the interaction of the chemical substances excreted by the mycelia concerned.

Inter-mycelial reactions correlated with sex[1] in the Hymenomycetes.

Again, it is possible that physiological differences, other than sex, may be determined by the genetical constitution of the haplonts concerned, in a manner analogous to sex heterothallism in the Hymenomycetes. Sex in this group of fungi is based on certain factors carried by the haplonts, which segregate out on Mendelian lines.

A factorial scheme for sex heterothallism was first worked out by Kniep (1920) in *Schizophyllum commune*, then more fully in *Aleurodiscus polygonius* (1922). In *A. polygonius* Kniep was able to isolate all the four spores from a single basidium.

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[1] The terms sex and sex heterothallism are retained in the sense in which they were originally used by the authors of the various papers to be discussed, but recent researches in *Humaria granulata* (Gwynne-Vaughan and Williamson, 1930) tend to support the view that the factors controlling fusion in the heterothallic Hymenomycetes are not sex factors but self-sterility factors. This point will be dealt with more fully in the discussion at the end of this paper.

Sex heterothallism has since been demonstrated by different workers in a number of other Hymenomycetes, more especially in the genus *Coprinus*, and it is now known that the same genus can contain both homothallic and heterothallic species. Heterothallism may be either simple or bi-polar, with only two genotypically different kinds of spores; or more complex or quadri-polar with four different kinds of spores. From his results in the quadri-polar *Aleurodiscus*, Kniep concluded that sex is determined by two pairs of factors. His results proved that no conjugation is possible between haplonts with one factor in common, but only between haplo-mycelia which differ in both factors.

In the quadri-polar species the spores of a single basidium may be of two kinds only (*A. polygonius*), but on account of the occurrence of di-hybridism the segregation of the sex factors may be different in different basidia, resulting in four kinds of spores in the same fruiting body; or, there may be complete segregation into four kinds of spores all geno-typically different in the same basidium, together with di-hybridism in other basidia of the same fruiting body, as demonstrated by Hanna (1925) and Newton (1926) in *Coprinus lagopus*.

It is somewhat misleading to apply the term sex to the four different haplonts, and to assume that there are four, or possibly more than four, sexes in the Hymenomycetes. A much simpler and more workable hypo-thesis is the conception of two sexes, the inter-reaction of any two given haplonts being controlled by their genetical constitution. These con-trolling factors are held by Kniep to be positive sex factors, by Prell (1921) and Brunswik (1924) negative sterility factors. On Brunswik's hypothesis, since all the mycelia from one fruiting body will show com-plete fertility with all the mycelia from another fruiting body, they must all be potentially bi-sexual, although haploid, and the lack of fusion between certain given mycelia from the same fruiting body is due to the effect produced by one self-sterility factor, or by certain combinations of two self-sterility factors. Brunswik also points out that the conception of one or two self-sterility factors not only explains the bi-polar and quadri-polar schemes, but also eliminates the necessity for the assump-tion of multiple allelomorphism to account for the complete inter-fertility between haplonts from different fruiting bodies, found in some Hymenomycetes.

So far as is known to the author, only one case has been recorded in the quadri-polar Hymenomycetes (*i.e.* with two sterility factors) in which failure to fuse is accompanied by the phenomenon of aversion, namely *C. micaceus*. In this species Vandendries (1929 *c*) found that

some geographical strains show clear cut tetrapolarity, in others this tetrapolarity is masked and the sexual affinity between the haplonts very variable. In contra-distinction to many other Hymenomycetes, inter-sterility between geographical races appears to be fairly common in *C. micaceus*, more especially between races from widely distant localities, and this failure to form clamp-connections is sometimes accompanied by definite aversion ("barrage") between the colonies. In certain geo-graphical races this "barrage" was also found between haplonts from the same fruiting body. As far as can be seen from Vandendries' tables, races which show *intra*-fruiting body aversion are capable also of showing aversion to haplonts from other races, and when haplonts from two such races are combined the number of cases of "barrage" is considerably increased. His complex results suggest that over and above sexual tetra-polarity the inter-reactions of the haplonts are influenced by some other sterility factors (*e.g.* factors for *inter*- and *intra*-racial aversion) segre-gating independently of sex. *C. micaceus* appears, however, to be in an unstable condition, and Vandendries is of the opinion that "toute per-turbation trouve sa raison d'être dans des mutations. Celles-ci sont in-dépendantes de facteurs extrinsiques, tel que climat, latitude, habitat, agents extérieurs."

In the bi-polar species (*i.e.* one sterility factor) Vandendries found aversion between haplonts of the same sex in *Paneolus complanatus* (1923) and *Coprinus radians* (1925 b), and Brunswik in *C. fimetarius* (1924 c, p. 64) and *C. Friesii* (1924 c, p. 28). In *P. complanatus* and *C. radians* like will not meet like, and aversion is intimately correlated with sex; but *C. Friesii* is especially interesting in that haplonts of the same sex may or may not show aversion "eine deutliche sichtbare Rille" towards one another. In the majority of cases they do, but not in all. Brunswik makes no further comment, but his results suggest the segre-gation of a factor for aversion carried by some haplonts and not others, a factor which can only bring about aversion when the haplonts are of the same sex.

As will be seen later, in *Diaporthe* like will meet like, but certain hap-lonts of certain constitution will also meet.

Mounce (1929) working on *Fomes pinicola*, a heterothallic species, tested seventeen cultures from ten different species of hosts, from the same and different localities. She found that colonies in the diplo-phase mostly showed mutual aversion, with only a few exceptions; also that "complete intermingling of mycelia occurred when the two inocula came from the same culture."

It appears that this aversion between the colonies in the diplo-phase, even from the same variety of host, is very general. In a table giving all possible combinations between thirty-five different cultures from *Picia mariana*, only a few cases of intermingling occurred and they were between closely related mycelia, such as those derived from the mycelium in the wood and the sporophore from the same tree; from sporophore tissue and spores from the same sporophore; or two sporophores from the same tree. Aversion was also found between mono-spore mycelia (haploid) accompanied by varying degrees of pigmentation of the hyphae from yellow to black along the line of contact, even from the same sporophore. Some of the mycelia, however, would meet and form clamp-connections, but showed a certain amount of pigmentation along the line of contact, although the intenser black colour never appeared between colonies forming clamp-connections.

From the results described in Mounce's paper, there appears to be no correlation between sex and aversion in *Fomes pinicola*, since aversion occurs between haploid mycelia and mycelia in the diplo-phase. Variations of light and temperature do not inhibit aversion, but the line of demarcation becomes more accentuated with the increase of nutrients in the medium. Varying degrees of aversion were also found.

Haplo-heteroecism in the Ascomycetes.

Although the essential cytological criteria of sex, namely nuclear fusion and the subsequent meiotic divisions, with or without a reduction in the number of chromosomes, have long been known and described by various workers in many species of Ascomycetes, haplo-heteroecism in this large group of fungi has only been demonstrated experimentally within recent years. Edgerton, in 1912–14, obtained plus and minus strains in *Glomerella*, but the differentiation of the sexes was not absolute, as monospore mycelia were capable of forming a few perithecia. Dodge in 1920, working with *Ascobolus magnificus*, found the first clear cut case of haplo-heteroecism, the development of perithecia being dependent upon the combination of two properly chosen monospore mycelia.

Further cases of simple haplo-heteroecism in the Ascomycetes are known: in *Neurospora sitophila* (Dodge, 1927 b; Wilcox, 1928), in which the sexes occur in approximately equal numbers among ascospores isolated at random; in *A. carbonarius* (Betts, 1926) and *N. crassa* (Shear and Dodge, 1927), in these two species both sexes have been found to occur in the same ascus in the ratio 1 : 1; in *Penicillium luteum* (Derx, 1925, 1926) where in promiscuous isolations half the number were of one

sex and half the other; in *Fusarium (Giberella) moniliforme* (Wineland, 1923, 1924)—in both *P. luteum* and *F. moniliforme* sterile ascocarps and perithecium-like structures respectively have been found in mono-spore cultures but they contained no ascospores; in *D. batatis* (Harper and Field, 1913), and *Ceratostomella paradoxa* (Dade, 1928).

Another doubtful case of haplo-heteroecism, accompanied by the formation of pigment along the line of junction between the mycelia of opposite sexes, has been recorded by Kirby (1923) in *Ophiobolus careceti*, but Davis (1925) was unable to confirm Kirby's results.

An interesting case of sex has been worked out by Shear and Dodge (1927) in *N. tetrasperma*. In this species the asci are normally four spored and the fungus haplo-synoecious. The number of spores in an ascus, however, may vary from one to four and sometimes there may be as many as six. In an ascus containing more than four spores the extra number is brought about by a further division of one or more of the spores. Shear and Dodge were able to isolate these smaller spores and found that they gave rise to haplo-heteroecious mycelia of two kinds. The larger normal spores contain two nuclei, presumably of opposite sexes, the smaller uni-sexual spores only one nucleus.

Aversion in the Ascomycetes.

A few cases of *intra*-specific aversion between mycelia of the same fungus have been recorded in the Ascomycetes.

Faris (1913) found aversion between mono-conidial mycelia of *Glomerella nephrolepis*.

Nakata (1925 *a*, *b*) records aversion between geographical strains of *Sclerotium Rolfsii* from different localities. In *S. Rolfsii* it appears that aversion between mycelia can be mutual or one-sided. When one-sided, one mycelium shows aversion, the other remains indifferent. As in *Diaporthe*, colonies from inocula from the same averting strain intermingle quite freely. Nakata concludes that when aversion occurs, the inocula are of different strains, when absent, they are of the same strain: also that "aversion is not due to a substance formed by the action of the fungus on the medium, but by a substance which is excreted by the mycelia irrespective of the medium." The original paper is in Japanese, and no mention is made of mono-spore cultures in the English abstract. Nakata only found the perfect stage (Pt III, *Hypochnus centrifugus*) in three strains out of a total of thirty-three from widely different localities. So far as is known only sclerotia and mycelium were used as inocula. Hence, presumably, the colonies were in the diplo-phase. He observed

fusion between hyphae of the same strain giving rise to the bi-nucleate condition (Pt VI) and also figures unstained clamp-connections, but as the mycelia did not originate from single ascospores, haplo-synoecism cannot be assumed on these data.

Aversion occurs only between biologic diplonts, and has not been demonstrated between haplonts, and may have nothing to do with sex in this fungus.

These two forms of aversion, between diplonts on the one hand and haplonts on the other, may only differ quantitatively, but there is always the possibility of their being due to entirely different causes.

As early as 1892 Reinhardt observed *inter*-specific aversion in the genus *Peziza*. When certain species of *Peziza* are combined, the normal elongation of the hyphae is arrested in the region of contact of the colonies. In other species combinations, some hyphae on meeting grow over into the opposite colony and coil round the opposing hyphae, and eventually both succumb. *P. sclerotiorum* and *P. trifoliorum* will both grow into a colony of *P. tuberosa* and kill the hyphae along the line of contact. When *P. sclerotiorum* and *P. trifoliorum* are combined, both mycelia intermingle at first, then in an area of the line of junction one species gains the upper hand, in another area the other, resulting in a zigzag line of demarcation.

Aversion in other groups of fungi.

In the haplo-heteroecious Phycomycetes Schmidt (1925) observed that in certain species mono-spore mycelia of the same sex showed aversion, and that the width of the line of demarcation was more or less correlated with distance apart of the inocula. *P. Blakesleeanus* +, *Mucor hyemalis* + and *M. mucedo* + all show mutual aversion. The width of the line of demarcation increases with the distance apart of the inocula, but decreases with the increase in concentration of the nutrient medium. Schmidt is of the opinion that in the Phycomycetes the arrest of growth at the point of contact of two mycelia is the result of the production of by-products of growth. He also found *inter*-specific aversion, and points out that Boas' hypothesis (1919), namely that aversion (Hemmungsraumbildung) is an indication of relationship, does not hold good for the Phycomycetes. Boas based his hypothesis on his observations on *Oidium lactis*. In this fungus *intra*-specific aversion frequently appears, whereas colonies of different species intermingle.

In *Saprolegnia* Müller (1922) is of the opinion that aversion is not due to depletion or lack of food substances, but thinks that certain definite

substances are excreted by the mycelia during metabolism. When peptone is the only source of C and N supplied, the line of aversion widens with the higher concentrations in the medium.

The nature of the various types of aversion in the Phycomycetes and their possible causes are fully discussed in Schmidt's paper, and need not be gone into more fully here.

In the Fungi Imperfecti, Koch and Rumbold (1921) figure aversion between mono-conidial colonies of *Phoma insidiosa*, a parasitic fungus on *Sorghum*, but no comment is made in the letterpress. The authors, however, state that, given certain conditions, two kinds of colonies invariably occur in platings on conidia, even from the same pycnidium; the one form will produce pigment of varying intensity in the medium, and the other not. Platings of the two different strains are figured (Koch and Rumbold, 1921, Pl. X); the colonies from the non-pigmented strain show very marked aversion accompanied by profuse pycnidial development, whereas in the pigment producing form the majority of the colonies show aversion although in a lesser degree, together with one or two cases of intermingling. The perfect stage is not described and is probably not known, but the fact that both strains can occur in the same pycnidium, and both appear to be capable of showing aversion, is interesting.

It is impossible to make any general statement as to the phenomenon of aversion and its causes from the few instances at present known, which are scattered throughout very widely divergent genera of fungi. Aversion can occur between mycelia in the diplo-phase, and may be *inter-* or *intra-*specific; or in the haplo-phase, and may or may not be correlated with haplo-heteroecism; like may or may not meet like, and so on.

Nuclear fusion and reduction division in the Ascomycetes.

In the Ascomycetes in general, the ascus stage is held to be the sexual stage, resulting from a nuclear fusion, giving rise to the zygotic definitive nucleus of the ascus. A considerable mass of evidence has accumulated during the last thirty years to show that this nuclear fusion takes place. But as regards the subsequent three divisions of this nucleus there appears to be little uniformity as to when or how the actual reduction of chromosome number or valency occurs among the various species which have been cytologically investigated. In some species the chromosome number is uniform throughout all three divisions, as first observed by Harper in *Phylactinia corylea* in 1905. Harper assumes a change of valency of the chromosomes in the successive divisions. Some eight

different species of Ascomycetes are listed by Schultz (1927) in his paper on *Peziza domiciliana* as having the same number of chromosomes in all three divisions in the ascus. In *P. domiciliana* eight is the number throughout. In other species, such as *Lachnea stercoria* (Fraser and Brookes, 1909), the chromosome number is the same in the first and second divisions, but the reduced number appears in the telophase of the third division.

An interesting case is recorded by Tandy (1927) in *Pyronema domesticum*, in which the definitive nucleus is sometimes tetraploid sometimes diploid, but the nuclei at the end of the third division are all haploid "showing that in the case of nuclei which were diploid after meiosis a further reduction has taken place."

Finally there is the much discussed case of *Humaria rutilans* (Fraser, 1908). The definitive nucleus has 32 chromosomes (16 gemini). Fraser holds the first division to be heterotypic (reductional) as sixteen chromosomes appear after the first meiosis, but in the telophase of the third division there are only eight chromosomes, a further reduction to which the term brachymeiosis is given.

Unfortunately no experimental cultural work appears to have been done on fungi which are known to have a uniform number of chromosomes throughout, with the exception of *Pyronema confluens* (Claussen, 1912), and nothing is known as regards haplo-heteroecism or the correlation, if any, between sex segregation and chromosomal behaviour. In *P. confluens*, a haplo-synoecious species, although the actual number of the chromosomes is the same throughout, Claussen holds the first to be the heterotype division with a reduction in valency of the chromosomes.

A valuable addition to our knowledge as to the segregation of sex and other physiological characters has been made by Dodge (1929). He has been able to prove experimentally that the factors determining the type of conidia of *Neurospora sitophila* occurs at the second division in the ascus, while the factors for sex segregates at the first.

In a recent paper on *Humaria granulata* Gwynne-Vaughan and Williamson (1930) have described an extremely interesting and new form of heterothallism, not so far recorded in any other fungus. All the mono-spore mycelia bear oogonia or female organs, which in the absence of mycelial fusion with another thallus cannot function, but shrivel and die after going through the initial stages of the normal development of the apothecium. The mono-spore mycelia, all morphologically identical, fall into two definite groups, arbitrarily termed + and − strains; but fertile apothecia are only produced in combinations of a + and a − strain. The

authors point out that this is not a case of sexual heterothallism, but suggest that "the condition may be tentatively described as nutritive heterothallism, but we have not been able experimentally to justify this term," and that "the thalli are either monoecious or of the female sex."

If this is a case of nutritive heterothallism, a further assumption has to be made to account for the sterility shown by mycelia of the same group. There is, however, another possible explanation for this peculiar and interesting form of heterothallism. No antheridium has been found; the mycelia are all identical and morphologically female, and when grown alone remain sterile; yet when combined half the number of combinations prove fertile. There must, therefore, have been mycelial fusion and interchange of nuclei at some stage after the colonies had met. This indicates that the mycelia are haplo-synoecious but self-sterile. It is to be assumed that this sterility is based on one self-sterility factor Aa, then this factor and its allelomorph would segregate at meiosis in the ascus, and half the spores would receive A and the other half a. The monospore mycelia from such a zygote would be of two kinds and give 50 per cent. of fertile combinations. Stated briefly this may be a form of physiological heterothallism based on one self-sterility factor in a haplo-synoecious fungus.

This valuable addition to our knowledge throws considerable light on the vexed question as to whether the factors controlling fusion in the Hymenomycetes are positive sex factors or negative sterility factors. The complete inter-fertility between many geographical races would be adequately explained on the assumption that the mycelia are all haplosynoecious, but heterothallic as regards one self-sterility factor in the bipolar species and two such factors in the quadri-polar. Vandendries' complex results suggest that there may be *inter*-racial sterility factors also influencing the inter-reactions between certain geographical races in *Coprinus micaceus*.

Thus heterothallism in the Hymenomycetes may not be due to sex or sex factors, but, as in *Humaria granulata*, may be a form of heterothallism based on one or more self-sterility factors (and possibly in some cases *inter*-racial sterility factors) in a haplo-synoecious species; and further, that the so-called homothallic species only differ from the heterothallic in that they carry no self-sterility factors.

DIAPORTHE PERNICIOSA (Marchal).

Life history, technique and behaviour of monospore cultures.

D. perniciosa grows well on various artificial media but requires at least six to nine months to complete one life cycle, thus restricting, more

especially in the early stages of this investigation, the choice of parents for the succeeding generations to be described below.

After preliminary tests had shown that aversion was not affected by the constitution of the medium, Quaker oats agar was used throughout for the aversion tests, and the fungus grown on sterilised plum twigs for the production of perithecia. The La Rue method (1920) of single spore isolation was adopted for all the spore isolations. The various stages of the life history of *D. perniciosa* have been described elsewhere (Cayley, 1923 *a, b*) and need only be briefly summarised here.

There are two distinct stages in the life history: (1) the pycnidial or vegetative spore stage, the pycnidia containing both oval "a" and filiform "b" spores, the "a" spores only being viable; (2) the perithecial or sexual stage, the perithecia containing numerous asci with eight bicellular ascospores and very evanescent paraphyses. These paraphyses were not recorded in the previous communications; being evanescent they were overlooked in the first stages of the investigation.

The perithecium arises from a much coiled multicellular archicarp the cells of which are multinucleate, but no male organ or nuclear fusions have been seen. It is difficult to trace the entire coil of the archicarp and in some instances there appeared to be more than one coil. Thus the male organ may also be a scolecite but indistinguishable in the coil mass. On the other hand, the perithecia as a rule are found in closely packed groups, and each coil may give rise to a perithecium.

One of the chief difficulties throughout this investigation was the sparing development of perithecia in pure culture. Polysporous pycno- and ascospore cultures produce pycnidia very readily but rarely perithecia. In nature also, the number of perithecia is few as compared with the excessive development of pycnidia on trees affected with "die-back." This led to the supposition that *D. perniciosa* might be a conglomerate of strains varying in their capacity for producing perithecia. A number of mono-pycnospore isolations were made, and in one petri dish sown with three single spores, one of the resulting mycelia showed aversion to the other two. The spore suspension from which these isolations were made was derived from more than one pycnidium. The pycnidia were quite typical and contained oval and filiform spores. Plate I, fig. 7, shows the original plate, fig. 8 shows a repetition of the same combination of cultures seven months later. The averting colony was labelled SS_1, and it is from that single colony that all the subsequent mono-spore isolations dealt with in this paper were derived.

During the course of the investigation several hundred mono-

pycnospore cultures have been made at various times within this series, and also from other sources, but the culture SS_1 was the only mono-pycnospore culture to produce perithecia from which it was possible to obtain ascospores for the succeeding generations. Two other pluri-pycnospore cultures developed perithecia, but were not tested.

Mono-pycnospore mycelia from the same pycnidium do not show aversion *inter se*, but can, and mostly do show complete aversion toward mycelia from different sources.

No explanation can be offered as to this exceptional case of a mono-pycnospore culture producing perithecia. To say it is a mutation, a statement for which there is no proof, is only an unsatisfactory way of shelving the question. The culture SS_1 was recorded as a single pycnospore isolation, but there is always the possibility of a dual origin from two pycnospores, although the utmost care in spore isolation has been exercised throughout. The pycnospores are small, $7 \cdot 5 - 9 \mu \times 2 \cdot 5 - 3 \mu$, colourless and somewhat difficult to find on a gelatine or agar plate; SS_1 was among the first few isolations made, very delicate skill in manipulation had not, as yet, been acquired, and another spore might have been included inadvertently.

SS_1 showed extra vigour from the first, the young colony was larger than the other two of the same age in the same petri dish, but the colony was circular and not lobed, as might be expected with the mycelia of two spores growing together.

Whether SS_1 is of single or dual origin is not of any great moment as far as the inheritance of aversion itself is concerned, but since evidence is now forthcoming that some strains (if not all) of *D. perniciosa* are haplo-heteroecious, it is as well to bear in mind that there is some possibility that SS_1 may not have originated from a single spore.

Sectoring in mono-ascus cultures.

Another complication has arisen during the course of this investigation. So far it has not been possible to isolate all the eight spores from a single ascus. In order to find out whether all the spores of an ascus behave alike, mono-ascus (*i.e.* whole ascus inoculum) as well as mono-ascospore isolations were made from the same perithecium. Tests for aversion with young mono-ascus cultures gave consistent results, they interacted with each other and with the rest of the sister mono-ascospore mycelia in the same way as any of the other cultures from the same perithecium. Later, however, after the cultures had been growing for some time and had been repeatedly sub-cultured, further aversion tests gave very conflicting re-

sults. The doubtful cultures were then grown singly in petri dishes, and were found to be throwing sectors. The sectors were marked off from the parent colony by a definite line of aversion. When tested with mycelia of known constitution, the sectors gave the reverse reaction to the parent colony (Plate I, figs. 5, 6).

This sectoring was not promiscuous, giving rise to a number of new saltants, as found by Brown (1926) in *Fusarium*, or "ever saltating" as the strain of *Diaporthe* described by Horne and Das Gupta (1929), but is somewhat analogous to the sectoring observed by Rayner[1] in *Phoma radicalis*, in which only two forms invariably occur.

Sectoring in *Diaporthe* has up to the present only been found in mono-ascus cultures, and the sector cultures have shown no further or back sectoring to the parent type. Mono-ascospore cultures have all remained constant. This suggests that all the spores of an ascus are not the same, although it is surprising that the cultures remained constant as long as they did.

Sex segregation is known to occur before the formation of the asco-spore, and the recent researches of Dodge (1929) have shown that other factors can segregate quite independently of sex during meiosis. On the other hand, mycelial segregation or saltations are also known in the fungi, and sectoring in *Diaporthe* may be vegetative. This fungus, however, appears to be in a fairly stable condition, and has mostly given quite consistent clear-cut results, so that the more probable explanation of sectoring in mono-ascus cultures is that the spores of an ascus are not all alike, more especially as the sectors have all proved to be of the opposite type to the parent colony.

In the early stages of the investigation, only mono-ascus cultures produced perithecia with viable ascospores. Subsequently, when it was realised that *Diaporthe* might be haplo-heteroecious, a series of dual cultures was set up, in which a number of mono-mycelia were combined singly with one and the same mono-mycelium. All the mycelia were of known origin belonging to the same generation; they had been tested previously, and had shown no aversion towards one another. The results are given in the Tables A, B and C. These tables show that a considerable number of these dual cultures proved fertile and produced perithecia, others failed to do so. The controls CA 17 (a), CA 32 (a) and CA 55 (a) developed perithecia-like structures, but no ascospores were found.

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[1] Dr Cheverley Rayner read a paper before the Genetical Society describing this phenomenon of sectoring in *Phoma radicalis* on 24 November, 1924. The work is not yet published, but I am informed that it is in the press.

The dual cultures grew well, with one or two exceptions noted in the tables.

Segregation in the first ascospore generation (AG_1)[1].

As stated above, the capacity for showing aversion is inherited, and segregates out in the second and subsequent ascospore generations from the pycnospore culture SS_1.

The first generation has been recorded in the previous paper on Aversion (Cayley 1923 *b*), but is reproduced here (Chart 1) as the cultures in Series XIV A, indicated by arrows, namely **111** and **113**, were taken as parents for the next generation. Only these two cultures produced perithecia.

In this AG_1 the mycelia with two exceptions all showed aversion towards one another (in other words almost complete *intra*-perithecial aversion) whether the cultures originated from different perithecia, from single asci or from single ascospores. Cultures *117* and *118*, and likewise *131* and *132*, were taken from the extreme edges of lobes of the same colony respectively, as it was thought possible, on account of the lobing, that two spores might have been introduced inadvertently in the inoculum. However, as they met in both cases, they probably came from the same spore. The remaining two instances, namely the meeting of *110* with *131* and *116* with *119*, are real exceptions. They were tested several times, giving the same result.

Segregation in the second ascospore generation (AG_2).

Culture **113** and subsequently **111** produced perithecia. In all, the progeny of nine different perithecia from mono-ascus culture **113** were tested, comprising 155 mono-ascospore and mono-ascus cultures.

The results of tests for aversion between the AG_2 mycelia from three separate perithecia are tabulated in Charts 2, 2 *a*, and 2 *b*. The remaining six perithecia showed almost complete *intra*-perithecial aversion, as in Chart 3.

As will be seen, the manifestation of segregation within the same perithecium may or may not occur, but when it does occur (Series XIX A)

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[1] For the sake of brevity and clearness it has been necessary to devise a system of notation to represent the alternating generations and the type of spore from which they originated, as follows:

PG_1 and PG_2 = First and second pycnospore (or conidial) generation.

AG_1 and AG_2 = First and second ascospore generation.

113 = No. of culture in heavy type denotes a whole ascus culture.

109 = No. of culture *in italic figures* denotes a single ascospore culture.

the mycelia fall into two definite Groups. A number of these mycelia were derived from mono-ascus isolations, as indicated by the numbers in heavy type. The remaining cultures were mono-ascospore cultures,

CHART 1.

Series XIV A. MP.   Series XIV B. MP.
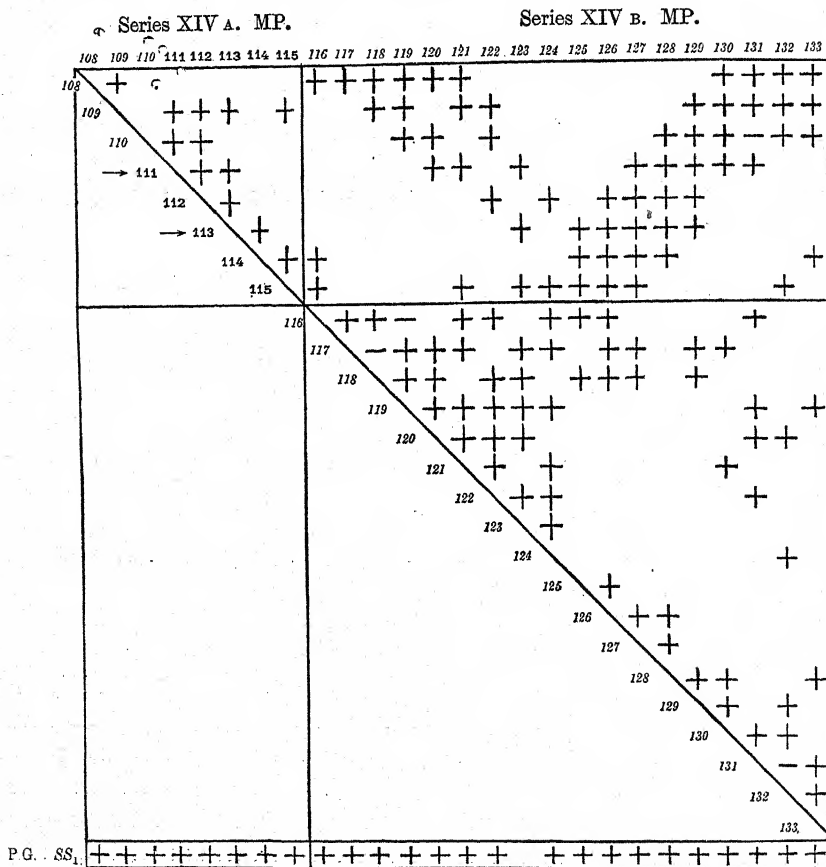
First ascospore generation (AG_1) from SS_1 showing general aversion.

113   Number in heavy type = whole ascus culture.
108   Number in italic figures = single ascospore culture.
+   Mutual aversion between the colonies.
−   No such aversion.
MP.   Spores from more than one perithecium.
→   Cultures used as parents for the next generation (see Chart 2).

or cultures from sectors thrown by different individual mono-ascus cultures at various times. The mycelia of one Group behave in the reverse way to the other Group as regards their inter-reactions, and the

parent belongs to one of the Groups. The original pycnospore culture SS_1, however, shows aversion to the AG_2 mycelia, irrespective of the Group to which they belong (Charts 2 and 2 a).

CHART 2.

Series XIX. SP.　　　　　XIX A. SP.　　　　　XIX B. SP.

	201	202	203	204	205	205 (b)	205 (d)	205 (e)	206	206 (a)	206 (c)	207	208	209	210	211	212	212 (a)	213
201		+	−	−	+	+	+	+	−	−	+	−	−	−	+	+	+	−	+
202	−		+	−	−	+	+	+	+	−	−	+	−	−	+	+	+	−	+
→ 203	+	+		+	+	−	−	−	+	+	−	+	+	−	−	−	−	+	−
204	−	−	+		+	+	+	+	−	−	+	−	+	+	−	+	+	−	+
→ 205	−	−	+	−		+	+	+	+	−	+	−	−	−	+	+	+	−	+
205 (b)	+	+	−	+	+		+	+	−	+	−	+	+	−	+	+	+	−	−
205 (d)	+	+	−	+	+	−		+	−	+	−	+	+	−	+	+	+	−	−
205 (e)	+	+	−	+	+	−	−		−	+	−	+	+	−	+	+	+	−	−
→ 206	+	+	−	+	+	−	−	−		+	+	+	+	−	+	+	+	−	+
206 (a)	−	−	+	−	−	+	+	+	−			+	−	+		+			+
206 (c)	−	−	+	−	−	+	+	+	−			+	−	+		+			+
207	+	+	−	+	−	+	+	+	+				+	+	−		+		−
208	−	−	+	−	+	+	+	+	+					−	+	+	+	−	+
209	−	−	+	−	−	+	+	+	+						+	+	+	−	+
210	+	+	−	+	+	−	−	−	+	+	−	+				+			−
→ 211	+	+	−	+	+	−	−	−	+	+	−	+	−				−	+	−
212	+	+	−	+	+							−	+	+				+	−
212 (a)	−	−	+	−	−							+	−				+	+	
213	+	+	−	+	+	−	−	−	+	+	−	+	+	−	+	+	−		
Parent 113	−	−	+	−	−	−	+	+	+	+	−	+	•	−	+	+			+
SS₁	+	+	+	+					+	+	+	+				+			+

AG_2. Progeny of AG_1 mono-ascus culture **113** showing segregation into two Groups, and their reactions towards

(1) the parent culture **113**;
(2) the original mono-pycnospore culture SS_1.

　113　Number in heavy type = whole ascus isolation.
　(a)　Letter enclosed in parenthesis = sector sub-culture.
　SP.　= single perithecium.
　→　Cultures marked with arrows = parents of the next generation (AG_3).

Other symbols as in Chart 1.

CHART 2 *a.*

Top matrix (Group I Haplonts):

	201	202	204	205	206 (a)	(c)	208	209	212 (a)	203	205 (b)	(d)	(e)	206	207	210	211	212	213	
201		−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	Group I
202	−		−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	Haplonts
204	−	−		−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	
205	−	−	−		−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	
206 (a)	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+		+	XYAb	
206 (c)	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+		+		
208	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+	+	+		XYAb
209	−	−	−	−	−	−	−		−	+	+	+	+	+	+	+	+	+	+	XYaB
212 (a)	−	−	−	−	−	−	−			+					+		+	+		xyAb
Group I 113	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	+	−	xyaB

Bottom matrix (Group II Haplonts):

	201	202	204	205	206 (a)	(c)	208	209	212 (a)	203	205 (b)	(d)	(e)	206	207	210	211	212	213	
203	+	+	+	+	+	+	+				−	−	−	−	−	−	−	−	−	Group II
205 (b)	+	+	+	+	+	+	+			−		−	−	−	−	−	−	−	−	Haplonts
205 (d)	+	+	+	+	+	+	+			−	−		−	−	−	−	−	−	−	
205 (e)	+	+	+	+	+	+	+			−	−	−		−	−	−	−	−	−	XYAB
206	+	+	+	+	+	+	+			−	−	−	−		−	−	−	−	−	
207	+	+	+	+	+	+	+			−	−	−	−	−		−	−	−	−	
210	+	+	+	+	+	+	+			−	−	−	−	−	−		−	−	−	
211	+	+	+	+	+	+	+			−	−	−	−	−	−	−		−	−	XYab
212	+	+	+			+	+			−	−	−	−	−	−	−			−	
213	+	+	+	+	+	+	+			−	−	−	−	−	−	−	−	−		
Group III 111	+	+	+	+	+	+	+			+					+	+		+	+	xyAB
Group IV 695	+	+	+			+				+	+	+	+					+	+	xyab
Group III 698	+	+	+			+	+	+		+		+	+	+		+				

Second ascospore generation from **113** arranged in Groups, and their reactions towards
    (1)   the parent culture **113**;
    (2)   Group III mono-ascus culture **111**;
    (3)   Group IV segregate *695* from **111** (see Chart 7);
    (4)   Group III culture *698* from **111** (see Chart 7).

CHART 2 *b.*

	Series XIX			XIX A.							XIX B.		
	201	202	203	204	205	206	207	208	209	210	211	212	213
Group I 113	−	−	+	−	−	+	+	−	−	+	+	+	+
SS₁	+	+	+	+	+	+	+	+	+	+	+	+	+

Reactions of cultures in Chart 2 before sectoring occurred.

It was among the mono-ascus cultures of this generation that sectoring occurred (Charts 2, 2 *a*). Culture **205** sectored three times, giving the three-sector cultures *205 (b)*, *(d)*, *(e)*: similarly culture **206** twice, giving *206 (a)*, *(c)*, and **212** once, giving *212 (a)*.

The sectoring parent cultures were kept sub-cultured and their reactions included in Charts 2 and 2 *a* (viz. **205**, **206** and **212**).

The earlier reactions of the above sectoring mono-ascus cultures are given in Chart 2 *b* where it will be seen that **205** meets its parent **113** and shows Group I reactions with the other mycelia in the series (Chart 2 *a*), but **206** and **212** show aversion towards **113** and give Group II reactions.

The number of mycelia in Series XIX and XIX B are too small to be taken as proof that three different kinds of perithecia are possible in one and the same mono-ascus culture, namely those which give:

(1) Progeny showing no visible segregation and no *intra*-perithecial aversion (Chart 2, Series XIX).

(2) Progeny showing no visible segregation but almost complete *intra*-perithecial aversion (Chart 2, Series XIX B; Chart 3).

(3) Visible segregation giving mycelia of two Groups (Chart 2, Series XIX A).

However, the results obtained from the tests of the progeny of these nine perithecia have definitely shown that certainly two kinds of perithecia can occur in the culture, those showing almost complete *intra*-perithecial aversion (2) (Charts 1 and 3), and those showing visible segregation (Charts 2, 2 *a*).

In Chart 3 it is interesting to note that the mycelia from perithecia showing almost complete *intra*-perithecial aversion not only show aversion towards mono-pycnospore cultures obtained from the parent colony **113** (vide SS_{125}) but also to the immediate mono-ascus parent **113**.

Several PG_2 mono-pycnospore cultures of **113** were used as tests, and they all behaved in the same way as the one culture (SS_{125}) recorded in Chart 3.

Also it will be seen in Chart 3 that two stock Group II cultures **203** and **211** (from the same culture) and of the same AG_2 generation, show aversion towards all other mycelia in the perithecium showing complete *intra*-perithecial aversion. The pycnospores of culture **113** show no *intra*-pycnidial aversion, in fact no certain case of *intra*-pycnidial aversion has been found in *D. perniciosa*. One doubtful case has occurred, but it was not possible to be quite certain that the spores tested came from the same pycnidium. All the remaining pycnidial tests from various sources and hosts never showed any *intra*-pycnidial aversion, but the mycelia

were capable of showing *inter*-pycnidial ʾaversion towards other pycno-spores from cultures from different hosts.

CHART 3.

Series XXXII. SP.
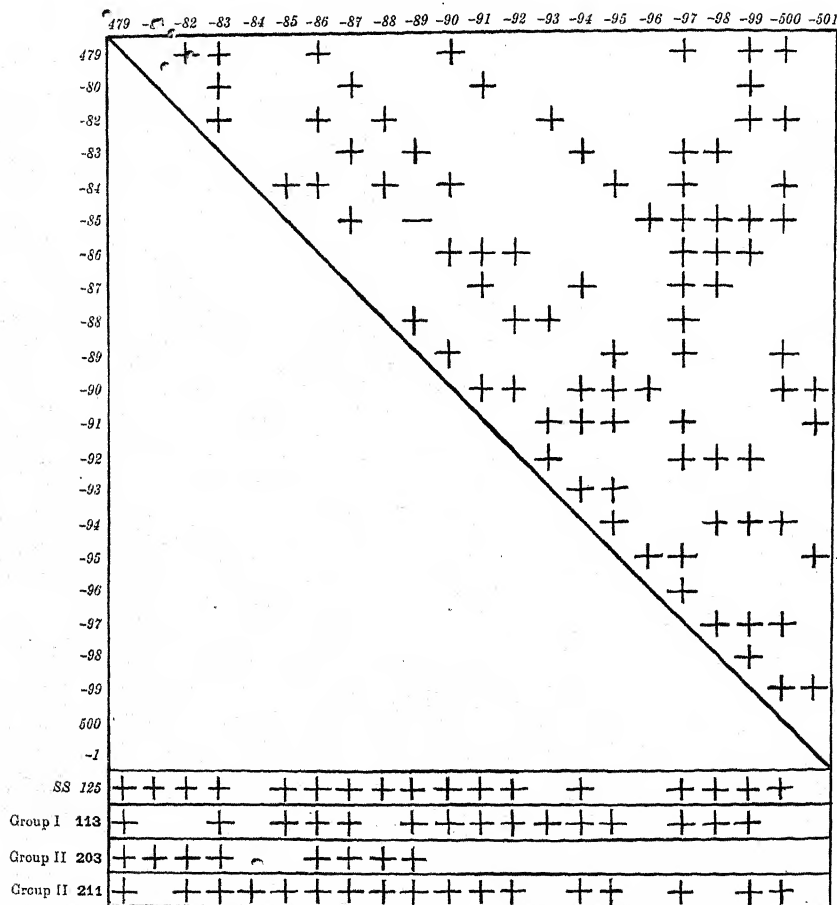
Progeny from a single AG_2 perithecium from Group I mono-ascus culture **113** showing general aversion, and the reactions of the mono-ascospore mycelia towards

(1) a mono-pycnospore culture from **113**, *i.e. 125*;
(2) the parent culture **113**;
(3) two Group II mono-ascus cultures, segregates from Group I **113**, **203** and **211**.

To summarise the foregoing results briefly before passing on to the next generation: *Mono-ascus culture* **113** *belonging to Group I* (*which was derived from a perithecium showing almost complete* intra-*perithecial aver-*

sion) has given at least two, and possibly three kinds of perithecia. The progeny of those perithecia giving complete intra-perithecial aversion all show aversion towards the immediate parent **113***, towards* PG_2 *pycnospore cultures of the same, and towards two different cultures of Group II of the same* AG_2 *generation. When visible segregation occurs some of the progeny will meet the immediate parent* **113***, others will not, and those which do not meet* **113** *give the reactions of mycelia of Group II. The mono-pycnospore culture* SS_1 *shows complete aversion towards its* AG_2 *mycelia irrespective of the group to which they belong. Mycelia of Group I can throw mycelia showing the reactions of Group II both in their progeny and by sectoring.*

Segregation in the third ascospore generation (AG_3).

Four mono-ascus cultures of the AG_2 developed perithecia, Nos. **203, 211, 205** and **206.**

1. From **203**, a Group II mono-ascus culture, 84 mono-mycelia were isolated from four different perithecia, one of which is recorded in Chart 4. The other perithecia gave similar results.

It will be seen that the majority of the mycelia show no aversion, but there are a few cases of incompatibility ($-$?) between some of the mycelia; this incompatibility generally disappeared in a few days, and at the time no great importance was attached to it; it was thought to be slight individual variation between the colonies.

All or most of the progeny met the parent culture, but three showed incompatibility, and all showed aversion to **113** and the original mono-pycnospore culture SS_1.

2. From **211**, a mono-ascus culture belonging to Group II, 71 mono-mycelia were obtained from four different sets of isolations from the same culture. In this case it was not possible to be quite certain that all the spores were obtained from single perithecia. The sterile twig on which they developed was much disintegrated, and as the perithecia were massed in clumps manipulation was difficult. Although a few spores from a neighbouring perithecium may have been included in the suspension of spores used for each set of isolations, certainly the majority of the spores originated from the same perithecium. The results of one of the four sets of isolations are tabulated in Chart 5, and arranged in their respective groups in Chart 5 a. The other three gave similar results.

Here again, as in the previous generation AG_2, the mycelia segregated into the two original Groups, giving eighteen Group I and thirteen Group II.

CHART 4.

Series *XXXV*. SP.   Series *XXXVI*. SP.

572 −73 −74 −75 −76 −77 −78 −79 −80 −81 −82 −83 −84 −85 −86 −87 −88 −89 −90 −91 −92 −93 −94 −95 −96 −97

Group II

Haplonts

XYAB

XYab

xyAB

xyab

Group II   203
Group I   113
  SS₁
Group I   205
Group II *205 (d)*

AG_3. Progeny of two single perithecia in Group II mono-ascus culture **203** a segregate from Group I culture **113**, showing no *intra*-perithecial aversion, but a few cases of incompatibility − ?; and their reactions towards

    (1) the parent culture **203**;
    (2) Group I culture **113**;
    (3) the original mono-pycnospore culture SS_1;
    (4) Group I mono-ascus culture **205** of the same generation as **203**;
    (5) Group II mono-ascus culture *205* (d) of the same generation as **203**.

    Cultures *576, 577* marked * were used as test cultures with the progeny of Group II culture **211** (see Chart 5).

CHART 5.

Series XXXIX. MP?.
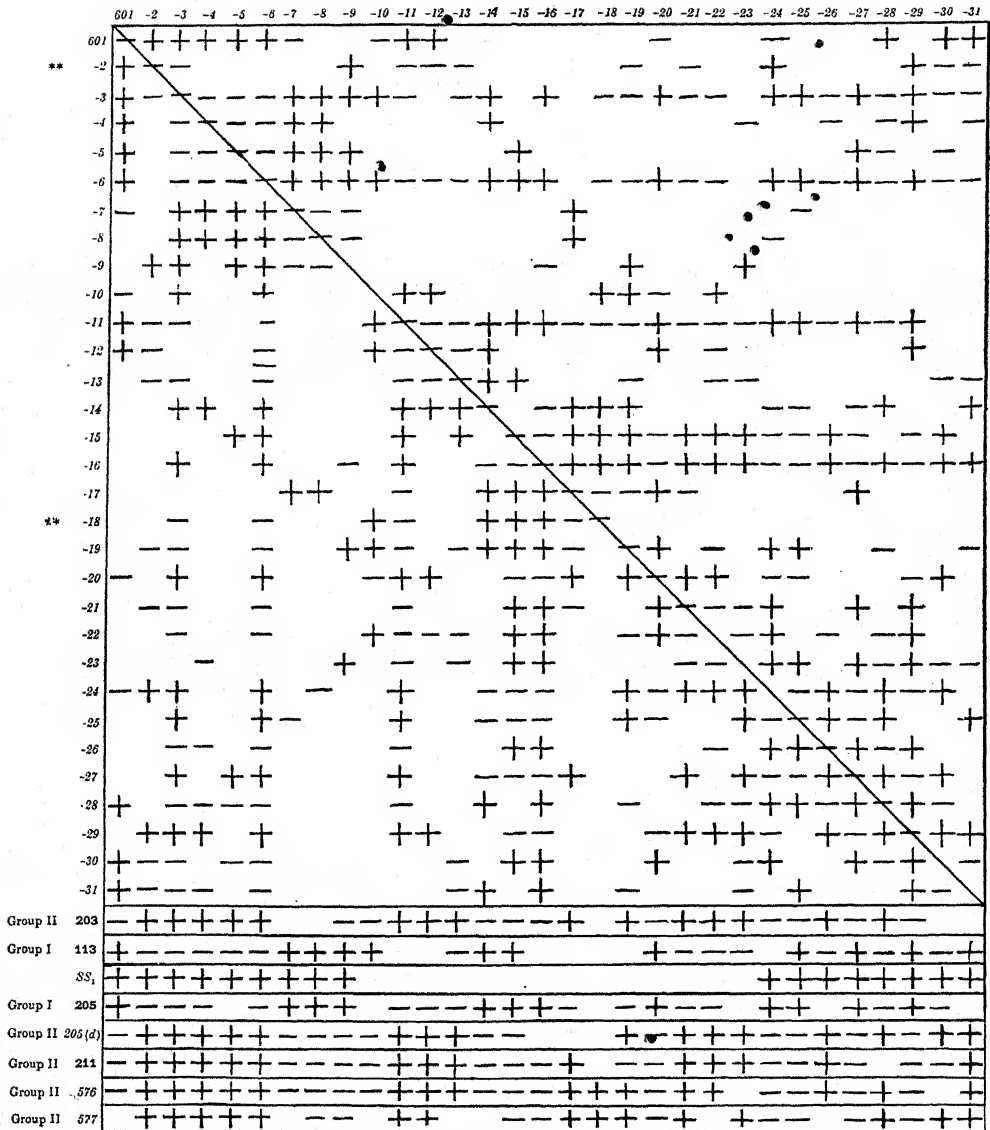
AG_3. Progeny from more than one (?) perithecium of Group II mono-ascus culture **211**, a segregate from Group I **113**, showing visible segregation into two Groups, and their reactions towards

(1) Group II mono-ascus culture **203**, of the same generation as the parent **211**;
(2) AG_1 Group I parent **113**;
(3) the original pycnospore culture SS_1;
(4) Group I mono-ascus **205** from the same generation as **211**;
(5) Group II sector culture from a Group I mono-ascus culture of the same generation as **211**;
(6) the parent culture **211**;
(7-8) two Group II mono-ascospore cultures from **203**, *576* and *577* (see Chart 4).

CHART 5 a.

Series XXXIX. MP?.
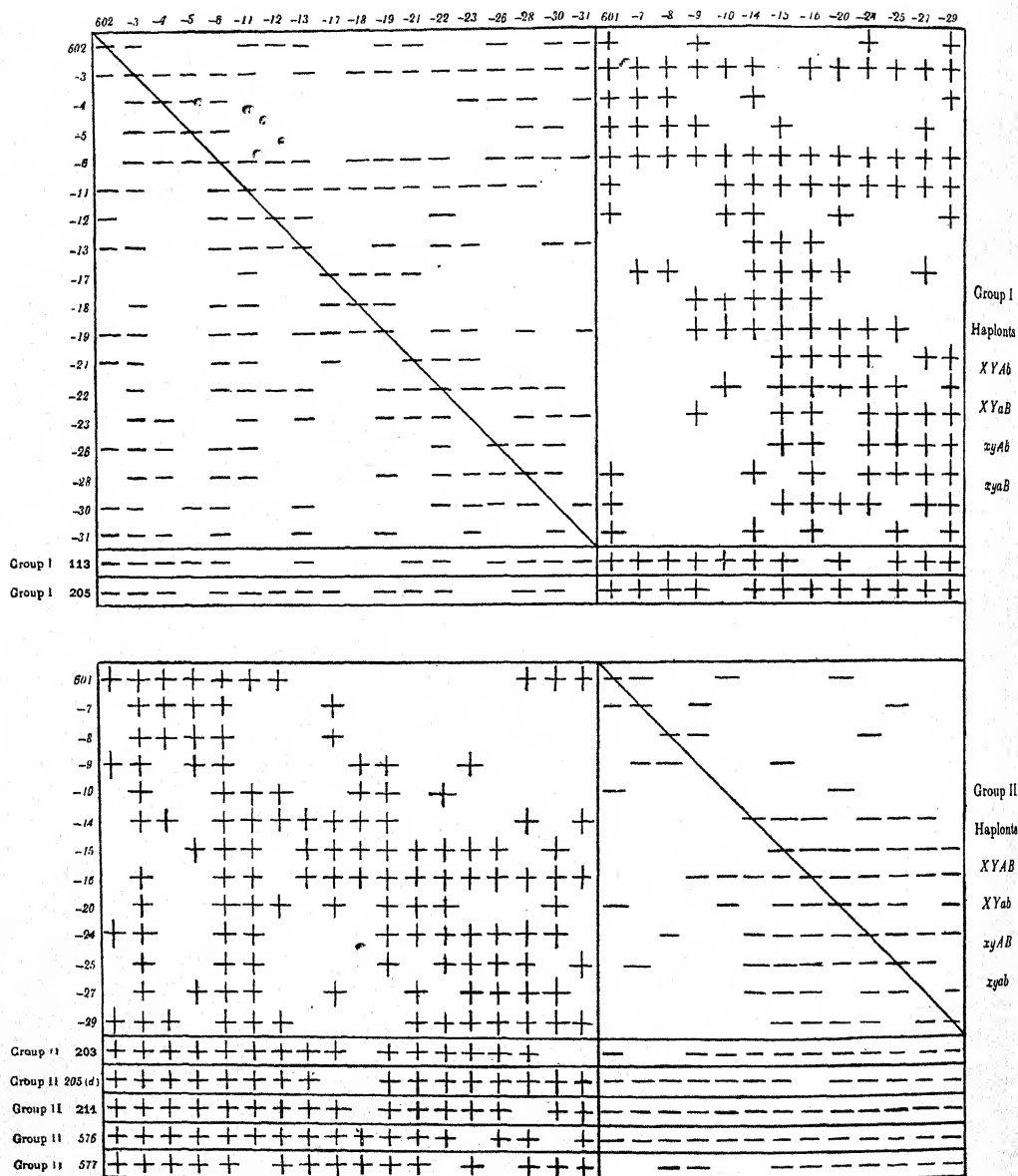
The same series as in Chart 5 arranged in Groups.

The mycelia of Group II can throw mycelia belonging to Group I in the same way as mycelia of Group I can throw mycelia belonging to Group II; but **211** has never given sectors of the opposite group.

Some of the mycelia in this series meet the immediate Group II parent **211** and other Group II cultures with which they were tested. Some of the test cultures used were from the same generation, others from the AG_2 (*vide* **203**, *205 (d)*), and yet others from the progeny of **203**, a later generation (*vide, 576, 577*).

Other mycelia meet Group I **113** and another Group I test culture **205**; but, as in the previous AG_2 generation, all the mycelia of this AG_3 show aversion to the original SS_1.

Comparing Chart 4 and Chart 5 it will be seen that although the parent culture **203** on the one hand, and **211** on the other, both give Group II reactions, they appear to be either genetically different, or differ as to the type of segregation which has occurred in the asci of the perithecia formed by these cultures.

Thus the statement made in the previous paper on Aversion (Cayley 1923 *b*, p. 356), to the effect that "since one colony on a plate can show aversion towards two other colonies which meet, all three of which have been isolated from the same host, like must meet like, and unlike show aversion to unlike" no longer holds, since the above results have shown that the occurrence of aversion or no aversion, between any two mono-ascospore mycelia, depends upon the genetical constitution of the two haplonts concerned.

3. From **206**, a mono-ascus culture belonging to Group II (a sectoring culture), twenty-two mycelia from two separate perithecia were tested. All the progeny proved to belong to Group II, showed no *inter-* or *intra-*perithecial aversion (within the same culture) and behaved consistently as Group II towards other stock cultures from both Groups (Chart 6, Series XLII).

4. From **205**, a mono-ascus culture belonging to Group I (a sectoring culture), although it was only possible to obtain four mono-mycelia from one single perithecium, segregation into the two original Groups occurred (Chart 6, Series XLIII).

The two parents **206** and **205** of the Series XLII and XLIII belong to different Groups, and show aversion to one another (Chart 2), but nevertheless they give the same two types of segregation in their progeny, as in the previous cases of the two Group II cultures **203** and **211**. Moreover **206** and **205** were isolated from one and the same perithecium.

To sum up, the results of these AG_3 tests show: *that the asci from*

*different perithecia in the same culture may belong to the same Group but can give different types of segregation. They can either show visible segregation resulting in two kinds of mycelia (**211**, Charts 5, 5 a) or no visible segregation (**203**, Chart 4) in their progeny; that asci from one and the same*

CHART 6.

Series XLII. SP. **206.**   XLIII. SP. **205.**

	672	−73	−74	−75	−76	−77	−78	−79	−80	−81	682	−83	−84	−86
672		−	−	−	−	−	−	−	−	−	+		+	
−73	−			−	−	−	−	−	−	−		−	+	
Group II −74	−	−		−	−	−	−	−	−	−	+		+	
−75	−	−	−		−	−	−	−	−	−	+		+	
−76	−	−	−	−		−	−	−	−	−	+		+	
−77	−	−	−	−	−		−	−	−	−				
−78	−	−	−	−	−	−		−	−	−	+		+	
−79	−	−	−	−	−	−	−		−	−			+	
−80	−	−	−	−	−	−	−	−		−	+		+	
−81	−	−	−	−	−	−	−	−	−				−	
Group I 682	+	+	+	+	+	+			+			+	−		
Group II −83		−	−	−	−	−	−	−	−		+		+	−
Group I −84	+	+	+	+	+		+	+	+		−	+		+
Group II −86	−			−		−		−				+		
Group II 206	−	−	−	−	−	−	−	−	−	−	+	−	+	−
Group I 205	+	+	+	+	+	+	+	+		−		−		
Group II 203	−	−	−	−	−	−	−	−	−	−	+	−	+	−
Group II 211	−	−	−	−	−	−	−	−	−	−	+	−	+	−
Group I 113	+	−	+	+	+	+	+	+	+	+	−	+	−	+

AG_3. Progeny of **206** and **205**.

206 shows no visible segregation.
205 shows visible segregation into two Groups, and their reactions towards
    (1) their respective parents **206** and **205**;
    (2) Group II culture **203**;
    (3) Group II culture **211**;
    (4) Group I AG_1 culture **113**.

*perithecium although they may belong to different Groups (**205, 206**, Charts 2, 2 a) can also show visible segregation (**205**, Chart 6) or no visible segregation (**206**, Chart 6) in the next generation; that individual asci from a perithecium showing segregation into two Groups, can in the next generation either give mycelia showing no* intra-*perithecial aversion (**206**) or visible segregation into the two original Groups (**205**); and finally that almost complete* intra-*perithecial aversion found in the first ascospore generation from the original culture* SS_1 *and in some perithecia of the* AG_2 *has not occurred again in the generation.*

The second ascospore generation in a parallel series.

A parallel series of cultures was made with the progeny of a sister AG_1 culture to **113**, namely mono-ascus culture **111** (see Chart 1).

Two separate isolations were made, but here again manipulation was difficult, and it was not possible to be quite certain that all the spores were derived from the same perithecium. As with **113**, the AG_2 from **111** also showed visible segregation into two Groups, one the converse of the other, the parent belonging to one of the Groups; but, when the individual mycelia from either of the Groups derived from **111** were tested with mycelia from both Groups from **113** they all showed complete mutual aversion, irrespective of the Group to which they belonged.

The Groups from **111** are designated as Groups III and IV.

The original mono-pycnospore culture SS_1 was obtained from diseased plum, and another series of mono-ascospore isolations were made from perithecia of *D. perniciosa* occurring on diseased branches of the pear, var. Williams' Bon Chretien. Here also complete *intra*-perithecial aversion was found, but owing to the large number of cultures already under investigation within the series from SS_1 no further work was done on this set of isolations.

Up to this point in the investigation, all the foregoing results were obtained from mono-ascus parents for the successive generations, as, being haplo-heteroecious as well as heterothallic for aversion, only those cultures produced perithecia and moreover sparingly, allowing of very little choice.

By combining two specially chosen mono-ascospore mycelia of known origin, either segregates which meet, or mycelia from perithecia showing no *intra*-perithecial aversion, it was hoped that further light would be thrown on the problem, and lead to some definite scheme as to the inheritance of aversion, and the correlation, if any, between the two forms of heterothallism, sex and the capacity for showing aversion.

CHART 7.

Series XLIV. MP.
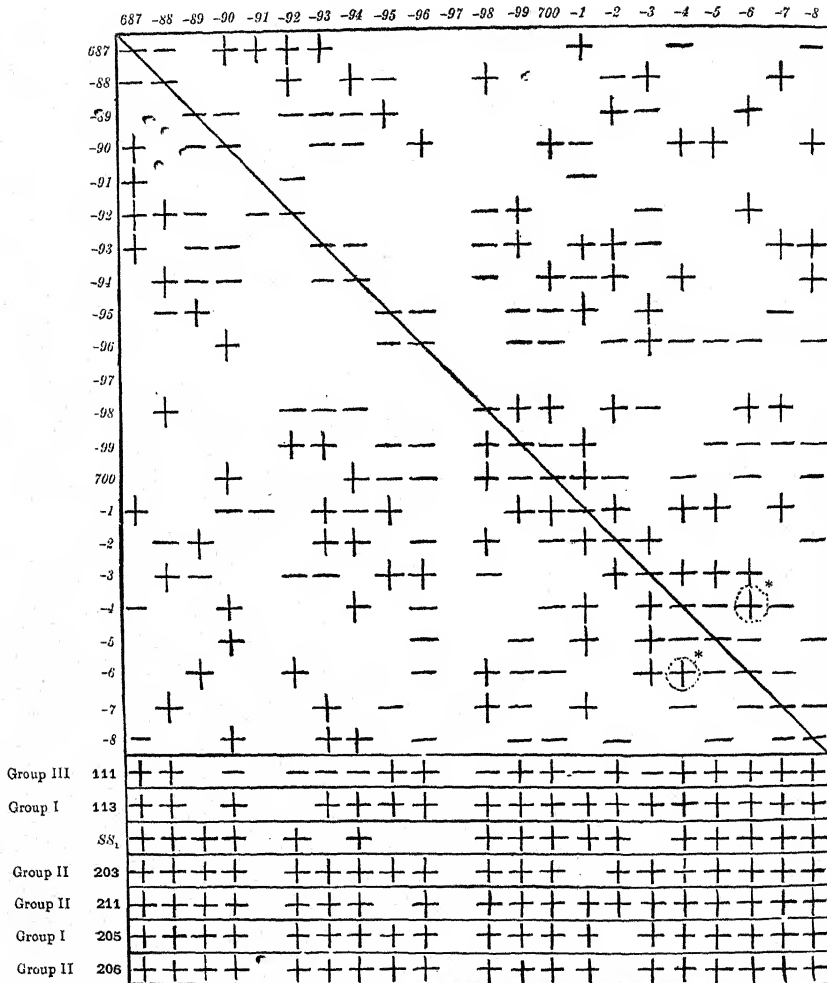
AG_2. Progeny of mono-ascus culture **111**, showing segregation into two
Groups III and IV, and their reactions towards

    (1) the parent culture **111**;
    (2) Group I culture **113**;
    (3) the original pycnospore culture SS_1;
    (4) two Group II cultures **203** and **211**;
    (5) Group I culture **205**;
    (6) Group II culture **206**;

MP.=spores from more than one perithecium.

⊕* Exception, tested several times giving the same result.

The tests, although incomplete, are suggestive, and the results so far as they go bear out the hypothesis that the two forms of heterothallism in *D. perniciosa*, aversion (which may be looked upon as a peculiar form of self-sterility) and sex, are not closely correlated, but segregate independently of one another. Also that *inter*-racial aversion (*i.e.* aversion

CHART 7 *a*.

Series XLIV. MP.
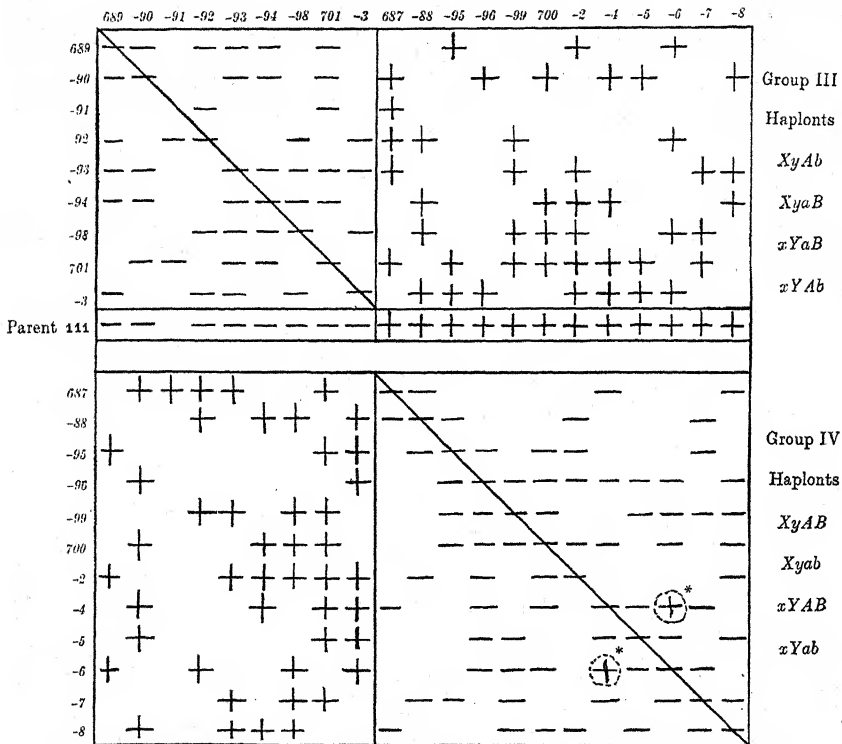
The same series as in Chart 7 arranged in Groups.
⊕* Exception, tested several times giving the same result.

between haplonts from different sources and hosts) and *intra*-perithecial aversion (*i.e.* aversion between haplonts from the same fruiting body, or different fruiting bodies in the same culture) are probably two distinct forms of aversion.

As pointed out in the introduction (p. 2) the haplo-synoecious form of *Diaporthe* was capable of showing *inter*-racial aversion but no *intra*-

perithecial aversion, and the haplo-heteroecious form discussed in this paper can show both.

These points will be gone into more fully when the results of the dual cultures have been considered.

Segregation and location of nuclei of different sexes and genetical constitution within the ascus.

Before going on to examine the results of the tests among the progeny of these dual cultures, the different ways in which segregation of sex can occur in the Ascomycetes and the distribution of the nuclei of different sexes among the spores of the ascus must be considered.

Our present knowledge of the segregation of sex in the ascus in haplo-heteroecious species is based on the few cases recorded in which the spores of a single ascus have been isolated, grown on and tested for sex, namely *Ascobolus carbonarius* (Betts, 1926), *Neurospora crassa* (Shear and Dodge, 1927), *N. sitophila* (Wilcox, 1928), and spp. of *Taphrina* (Wieben, 1927). In all these cases the sexes have been found to occur in equal numbers, four of one sex and four of the other. Wilcox's work on the distribution of the spores in the ascus of *N. sitophila* has shown that the distribution of the sexes can occur in four different ways, when the spores lie in a more or less linear series along the main axis of the ascus. They may either

(1) alternate in pairs in two different ways; or

(2) there may be four spores of one sex lying between the two terminal pairs of the opposite sex at either end of the ascus. This again can occur in two different ways.

Wilcox never found spores of different sexes alternating singly. Further, Dodge (1927 *b*) figures the position of the spores of each sex in a typical axis if segregation of sex factors should take place at the first division. In this case the four spores of one sex will lie at one end of the ascus and the four of the opposite sex at the other.

He was able to go a step further and bring cytological evidence to bear on the problem of the distribution of the nuclei during the three divisions in the ascus of *Neurospora tetrasperma*. As already stated this fungus is normally homothallic, the ascus is four-spored and the spores bi-cellular; also if a further but abnormal division of the bi-cellular spores takes place in the ascus, giving rise to uni-nucleate unicellular spores, these spores are uni-sexual and the resulting mycelia haplo-heteroecious. Thus the nuclei of the two cells of a normal bi-cellular spore are of different sexes, and "Homothallism and heterothallism in the genus *Neurospora* are not absolutely fixed specific characters, although the sexual nature of an

individual haplont is definitely determined by the time the spore is cut out."

The nuclei of *N. tetrasperma* are pear-shaped and have a beak-like appendage at one end, so that after any given division, the daughter nuclei and the halves of any individual nucleus can be easily distinguished. Their location depends upon the orientation of the spindles of the second and third division in the ascus.

The spindle of the first mitosis is longitudinal, parallel to the main axis of the ascus, but the orientation of the second spindle Dodge observed could be either oblique or longitudinal; that of the third nearly transverse. He was able to show that "regardless of whether segregation takes place at the first, second, or third division, each spore will contain one nucleus of each sex." For further details Dodge's paper should be consulted.

Since sex can be distributed amongst the nuclei of the ascus in these several different ways, it is justifiable to assume that other segregating characters or factors can be distributed in a like manner.

Dodge (1929) has been able to prove experimentally that the segregation of morphological characters such as the colour of conidia and general appearance of mono-spore colonies of *N. sitophila* can occur independently of sex segregation in the same ascus, the former takes place at the second and the latter at the first nuclear division of the ascus.

The ascus of *D. perniciosa* is eight-spored, and the spores are two-celled with a single nucleus in each cell. As the fungus is haplo-heteroecious both the nuclei of each spore must be of the same sex, but there is no evidence to show that the nuclei of each spore differ from one another in any other respect. They may or may not be the same.

DUAL CULTURES OF MONO-ASCOSPORE MYCELIA AND SEGREGATION IN THE FOURTH GENERATION.

Several sets of dual cultures were grown on sterilised plum twigs. In each set, one individual mono-ascospore mycelium was combined severally with various other mycelia, to which it had shown no aversion, either from the same perithecium or from other perithecia from different cultures belonging to the same group, or again with segregates derived from cultures belonging to the opposite group.

During the earlier stages of the investigation the reactions between the mycelia were mostly quite marked and easily recorded, and only those reactions which showed a very definite line of demarcation between the colonies were recorded as +. Occasionally a slight difference was

noted between free intermingling and signs of incompatibility, as in Chart 6.

Later, however, during the tests of the progeny of the dual cultures, the line of aversion was never so pronounced as in the previous generations, and varying degrees of aversion became evident. The recording of the somewhat indefinite intermediate degrees of aversion proved to be both difficult and unsatisfactory, more especially as the cultures appeared to be losing their virulence, either from prolonged culture or from close inbreeding. But when aversion tests were made between mycelia of the previous generations and the progeny of the dual cultures, the line of aversion was much more definite than between the mycelia from the dual cultures tested *inter se*.

The results of these tests cannot be gone into in detail here, as the tests are incomplete, and it is feared that the investigation will have to be abandoned for the reasons given above, but these somewhat indefinite results may prove of some value for further work on other species of Ascomycetes, more amenable to cultural conditions and with shorter life cycles.

The dual combinations and the mycelia used are set out in Tables A, B and C. All the mycelia in these several different tables of dual cultures are mono-ascospore mycelia of known origin within the series derived from **113**.

Table A comprises combinations between culture *602* (a Group I segregate from the Group II mono-ascus culture **211**) (see Charts 5, 5 *a*) with other segregates of the same Group from the same culture and possibly the same perithecium; in other words, this set of combinations CA 1–CA 17 (*a*) comprises mycelia derived from perithecia showing visible segregation in their progeny, and the mycelia chosen for these cultures are segregates belonging to the opposite group to the parent culture.

Out of 18 dual cultures eleven produced perithecia, a somewhat surprisingly high percentage of fertile cultures from a perithecium showing segregation. Nine of these cultures were dissected successfully, and the remaining two, although they produced perithecium-like structures, were probably sterile, as no asci or ascospores were found. Thus out of eighteen dual cultures nine were fertile, a ratio that might be expected if the sexes occur in equal numbers.

The control culture *602* grown by itself also developed what appeared to be normal perithecial necks, but when dissected out, the basal spherical portion of the perithecium, which should have contained asci, was undersized, difficult to find and filled with a mass of disintegrating tissue

rich in oil. This culture was dissected twice, very carefully, with the same result.

It must be pointed out that, although the above nine cultures proved to be fertile, very few perithecia as a rule were found in any one culture; and, as the manipulation was always somewhat difficult, the number of perithecia tested was necessarily small. In CA 5, for instance, which was dissected too young, only two or three perithecia could be found, and this culture failed to produce any more.

TABLE A.

Dual cultures of monospore mycelia, segregates belonging to Group I from Group II culture **211**. *(See Charts* 5 *and* 5 *a.)*

N.B. The progeny of **211** showed visible segregation and *intra*-perithecial aversion.

Haplonts combined	Culture no.	Perithecia	Results
602 & 603	CA 1	0	
604	2	+	4 perithecia dissected, 2 tested; varying degrees of aversion
605	3	+	Not tested, dissected twice without success, probably sterile perithecia
606	4	+	1 perithecium tested, no *intra*-perithecial aversion
607	4 (*a*)	0	
611	5	+	Not tested, dissected too young, immature asci; fertile
612	6	0	
613	7	+	2 perithecia tested, varying degrees of aversion, in one perithecium – reactions, in the other +reactions predominating
617	8	+	2 perithecia tested, no definite *intra*-perithecial aversion
618	9	+	4 different dissections, 3 from single perithecia, no *intra*-perithecial aversion
619	10	+	1 dissection, mixed spores from 2 perithecia in close juxtaposition, irregular segregation and varying degrees of aversion
621	11	0	
622	12	+	Not tested
623	13	+	Dissection unsuccessful; probably sterile
626	14	0	
628	15	0	
630	16	0	
631	17	+	2 perithecia tested, irregular segregation, varying degrees of aversion
602 alone	17 (*a*) Abortive		Dissected twice, no ascospores found

0 = no perithecia.   + = perithecia.

Three of these dual cultures in Table A, CA 4, CA 8 and CA 9 have given perithecia showing no segregation and no *intra*-perithecial aversion; others, CA 2, CA 7, CA 10 and CA 17 irregular segregation and varying degrees of aversion; but so far, none have given complete *intra*-perithecial aversion or segregation into the two original Groups. Since

all the mycelia used for this series of dual combinations were Group I
segregates, therefore Group I has proved to be capable of producing peri-
thecia showing no *intra*-perithecial aversion besides the two other types
of perithecia described on p. 19 and tabulated in Charts 2 and 3. On
the other hand, mycelia of Group II have only given two types of peri-
thecia, those showing visible segregation into two Groups, and those
showing no *intra*-perithecial aversion.

From the results set out in Table B, obtained from dual cultures of
Group II mycelia derived from **203** (Chart 4), it will be seen that out of

TABLE B.

Dual cultures of monospore mycelia belonging to
Group II from Group II culture **203**.

N.B. The progeny of **203** showed no definite *intra*-perithecial aversion but
a few cases of slight incompatibility. (See Chart 4.)

Haplonts combined	Culture no.	Peri-thecia	Results
576 & 577	CA 18	0	
578	19	+	Not tested, but asci containing immature ascospores found; fertile
579	20	0	
580	21	0	
581	22	+	2 dissections unsuccessful; probably sterile
582	23	0	Poor growth
584	25	+	1 perithecium tested, definite segregation into two Groups
586	27	0	
587	28	0	
588	29	0	
589	30	0	
590	31	0	
591	32	+	Dissection unsuccessful; probably sterile
576 alone	32 (a)	Abortive	Dissected twice, no perithecial bases or ascospores found

0 = no perithecia.   + = perithecia.

thirteen dual cultures only four produced perithecia. This low percentage
of fertile cultures is again rather surprising. As the parent culture showed
no definite *intra*-perithecial aversion and only a few cases of slight in-
compatibility, a much higher percentage of fertile cultures might have
been expected.

Of these four cultures which produced perithecium-like structures,
only two proved to be definitely fertile, CA 19 and CA 25. CA 19 was
dissected too young but definite asci containing immature ascospores
were found. Mycelia from spores from a single perithecium in CA 25
showed definite segregation into the original groups.

CA 22 was dissected on two separate occasions but no ascospores were
found, and the dissection of CA 32 was also unsuccessful.

These two sets of dual cultures in Tables A and B were derived from Group II mono-ascospore cultures from different perithecia in 113; both the parents belonged to Group II but the mycelia raised from the dual cultures in Table A were Group I segregates, and those in Table B belonged to Group II. Thus dual combinations of Group I mycelia have given perithecia with varying degrees of aversion together with other perithecia with no *intra*-perithecial aversion, and one dual combination of Group II has given definite visible segregation into the two original groups.

Again, the dual cultures in Table C comprise combinations between Group II mycelia from culture **206** (Chart 6), the one culture *673* being combined severally with other mycelia from the same perithecium. Two out of eight of these dual cultures have proved fertile, and both gave irregular segregation and varying degrees of aversion. Therefore Group II can also give perithecia showing this variability.

TABLE C.

Dual cultures of monospore mycelia from Group II culture **206**.

N.B. **206** showed no *intra*-perithecial aversion in its progeny.

Haplonts combined	Culture no.	Peri- thecia	Results
673 & *674*	CA 46	0	Poor growth
675	47	0	
676	48	0	
677	49	+	2 perithecia tested, varying degrees of aversion, with − reactions predominating
678	50	0	
679	51	0	
680	52	+	2 perithecia tested, varying degrees of aversion
681	53	0	
673 alone	53 (*a*)	Abortive	

0 = no perithecia.   + = perithecia.

DISCUSSION OF RESULTS.

This is the first record of experiments dealing with the inheritance of the capacity for showing *intra*-perithecial aversion in a heterothallic fungus, and, as can be seen from the results recorded above, the problem is far from simple, although the results themselves are fairly clear cut.

No complete elucidation of the problem can be offered from the data to hand, and as the cultures are losing their vitality, further extensive work would prove neither profitable nor reliable, on account of the difficulty in recording the varying degrees of aversion shown by weakened mycelia. Hence the results will be discussed as far as they go, and where possible, suggestions for a factorial scheme offered. The genealogical

Table D on pp. 56, 57 gives the results in brief of the four ascospore generations from SS_1 described in this paper.

The most striking points brought out by these experiments are: the inheritance and segregation of the capacity for showing aversion, the sterility of mono-ascospore cultures and the occurrence of definite groups of mycelia, which, although they cannot meet, can throw mycelia of the opposite group in their progeny.

Two forms of Aversion, inter-*racial and* intra-*perithecial.*

The results indicate that there are two forms of aversion, *inter*-racial aversion between biologic races, and *intra*-perithecial aversion between mycelia from the same fruiting body. These two forms of aversion are macroscopically indistinguishable. *Inter*-racial aversion may be looked upon as a form of sterility between biologic races, and *intra*-perithecial as self-sterility, a peculiar form of physiologic heterothallism other than sex.

It has been demonstrated experimentally that both the haplosynoecious and haplo-heteroecious forms of *Diaporthe* are capable of showing *inter*-racial aversion, but that *intra*-perithecial aversion has only been found in the haplo-heteroecious form.

Both forms of *Diaporthe* are found associated with "die-back" on different species of *Prunus* and on *Pyrus malus*, and races or strains of either of these forms mostly show *inter*-racial aversion. Two rather surprising exceptions have been found, however, namely mono-pycnospore mycelia of the haplo-heteroecious form isolated from apricot showed no aversion towards mono-pycnospore mycelia of the haplo-synoecious form from the plum Coe's Golden Drop; and again, mono-ascospore mycelia from two haplo-synoecious forms from two different varieties of plum showed no aversion.

Thus although *inter*-racial aversion is very general, some races will meet. These facts suggest that the biologic races that meet are either identical, or some races may carry the factor or factors for *inter*-racial aversion and others do not, or again, that races of certain given constitutions although carrying *inter*-racial aversion factors can meet. Also it appears as if the *inter*-racial aversion shown by both forms of *Diaporthe* may be due to the same cause.

No work has been done on the inheritance and segregation of sex in this fungus; but the results of other workers have shown that, in the Ascomycetes, the sexes occur in the ratio of 1 : 1 in the same ascus. The location of the nuclei of different sexes before spore delimitation has

been shown by Dodge (1927 b) to depend upon whether sex segregation takes place during the first or second meiotic division, and also on the orientation of the spindles of the second meiotic division and the third division, generally held to be mitotic.

Hence it is justifiable to assume that in *Diaporthe* also the sexes occur in equal numbers. The influence of *intra*-perithecial aversion on the fertility between mycelia of different sexes will be considered later.

Suggested factorial scheme for the inheritance of Aversion.

The different Groups of mycelia must be considered first, as a basis for further discussion on the more complex inter-reactions due to the two forms of aversion and sex. As to *intra*-perithecial aversion, the results have shown that there are at least two Groups of mycelia, and possibly four. Assuming that there are only two, then the fact that one Group will not meet the opposite Group, but yet can throw the opposite Group in its progeny can be explained on the hypothesis that there are two pairs of self-sterility factors *AaBb*. With certain modifications (as set out below) the inter-reactions due to *intra*-perithecial aversion factors fall into the scheme elaborated by Kniep (1920, 1922) for sex based on two factors in the Basidiomycetes, although the factors *AaBb* in *Diaporthe* are not sex factors. Kniep found that sexual fusion could only take place between haplonts with no factor in common, so that the only possible zygote is always heterozygous for both factors. This holds good as far as the actual formation of zygotes is concerned in *Diaporthe* also, and the only possible zygote is *AaBb*. But in this fungus, experimental results have shown that like will meet like, and therefore colonies of both homo- and hetero-haplonts when of the same genetical constitution will also meet (*e.g. AB* and *AB*, *Ab* and *Ab*, and so on), but nevertheless no zygotes are possible in such combinations of mycelia. Zygotes can only be formed between homo-haplonts of different genetical constitutions, *AB* and *ab*, and between hetero-haplonts with no factor in common, *Ab* and *aB*. Hence the only possible zygote is *ABab*.

Aversion only occurs between mycelia when the balance of the allelomorphic pairs is upset (*e.g. AB* and *Ab*, *Ab* and *ab*, and so on) as set out below:

(*a*) Aversion occurs in:

(1) Combinations between homo- and hetero-haplonts where the balance is upset

AB	*Ab*
AB	*aB*
Ab	*ab*
aB	*ab*

(*b*) No aversion in:

(1) Combinations of homo-haplonts of the same genetical constitution	*A B* *a b*	*A B* *a b*
(2) Combinations of homo-haplonts of different genetical constitution	*A B*	*a b*
(3) Combinations of hetero-haplonts of the same genetical constitution	*A b* *a B*	*A b* *a B*
(4) Combinations of hetero-haplonts with no factor in common	*A b*	*a B*

By analogy with what is known of segregation in both the Basidio-mycetes and Ascomycetes, it is justifiable to assume, from the data to hand, that in *Diaporthe* also segregation of factors other than sex can take place either at the first or second meiotic division of the zygote nucleus in the ascus.

If the factors *AaBb* segregate independently, then there are five pos-sible types of segregation which can occur during meiosis in an eight-spored ascus, as set out in Scheme I. This Scheme takes into account the genetical constitution of haplonts belonging to Groups I and II only and the inhibition of sexual affinity of the two sexes due to their genetical constitution.

Haplonts belonging to Group I are all hetero-haplonts of the con-stitution *Ab* or *aB* (Scheme I (1)), those of Group II all homo-haplonts *AB* or *ab* (2). This attribution of the factors to the two Groups is of course purely arbitrary. In terms of this hypothesis mycelia of Group I will always meet others of Group I, but will always show aversion (irrespec-tive of their genetical constitution) towards both kinds of mycelia of Group II, owing to the lack of balance of the allelomorphic pairs. But since the zygote formed by the fusion of haplonts within each Group is *ABab*, then the zygotes of either Group can throw mycelia of the opposite Group in their progeny, and the occurrence of true *intra*-perithecial aversion or no aversion in the next generation will depend upon the type of segregation which has occurred during meiosis in the asci of the parent culture.

Thus the segregation of sex and of the factors for aversion in the first (or possibly the second) division in Scheme I (1) and (2) will give mycelia of Group I only in (1), and Group II in (2), and there will be no *intra*-perithecial aversion, and thirty-two possible fertile combinations in both cases.

In (3) with di-hybrid bi-polar segregation of *ABab* in the second and

sex in the first division, some mycelia will show aversion and others not, and the mycelia will fall into two Groups, the Groups giving the reverse reactions. But since all the mycelia which meet are of the same sex, no fertile combinations are possible.

In (4) on the other hand, with sex segregation and di-hybrid bi-polar segregation of *ABab* both at the second division, sixteen fertile combinations would be possible, and the zygotes all *ABab*.

In (5) with sex segregation at the first division and Group I type of segregation of *ABab* at the second there will be only sixteen fertile combinations possible out of sixty-four. The same can be said of (6), only the mycelia would all be Group II.

There are of course further possibilities when sex and the factors for *intra*-perithecial aversion are segregating independently, but the cases given in Scheme I are sufficient to show how the hypothesis fits the experimental results recorded. Irrespective of whether sex segregates at the first or second division, the visible results will always be the same, either no *intra*-perithecial aversion, or if aversion occurs the mycelia will fall into two Groups in the ratio of 1 : 1. No case has been found of a fertile culture belonging to either Group giving rise to a perithecium throwing mycelia of the opposite Group only, although this is theoretically possible.

Thus, in cultures belonging to either Group, perithecia that have shown bi-polar segregation can give both bi-polar or quadri-polar (di-hybrid bi-polar) in the next generation. When purely bi-polar, as in Scheme I (1), (2), (5) and (6), the type of segregation is that of the parent culture, as neither Group has produced perithecia containing spores of the opposite Group only. Therefore, although the zygote nucleus formed by the meeting of mycelia of either Group is genetically the same, the Groups differ as to their bi-polar segregation.

On the other hand, the possibility of there being four Groups cannot be ignored. The behaviour of Groups III and IV towards Groups I and II could be explained in two ways. They may either be the same as Groups I and II respectively, but on account of some undetermined effect produced by *inter*-racial aversion they may be unable to meet; or, there may be four definite Groups all genetically different as far as *intra*-perithecial aversion factors are concerned. In the latter case two more pairs of self-sterility factors must be assumed. The experimental results can be equally well explained on a quadri-factorial scheme, and, as will be seen later, there are indications that more than two pairs of self-sterility factors are involved.

On the same assumption that the factors can segregate independently

SCHEME I.

Types of segregation of the intra-perithecial *aversion factors A Bab.*

(1)   ♂   ♀
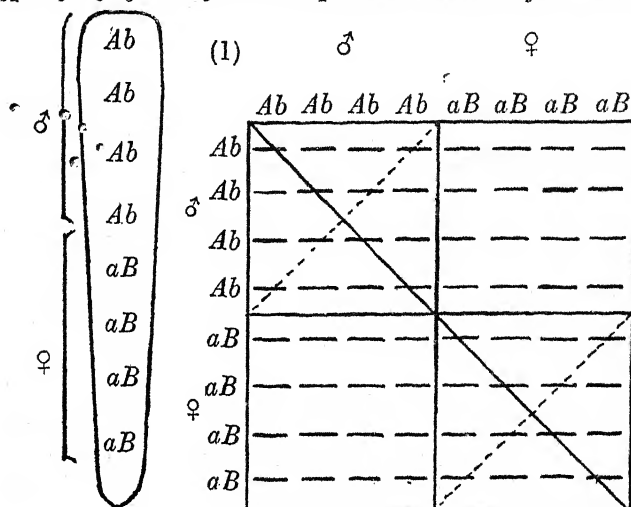
Sex and Group I segregation of *A Bab* at the
1st division.
All Group I mycelia.
32 fertile combinations possible.

(2)   ♂   ♀
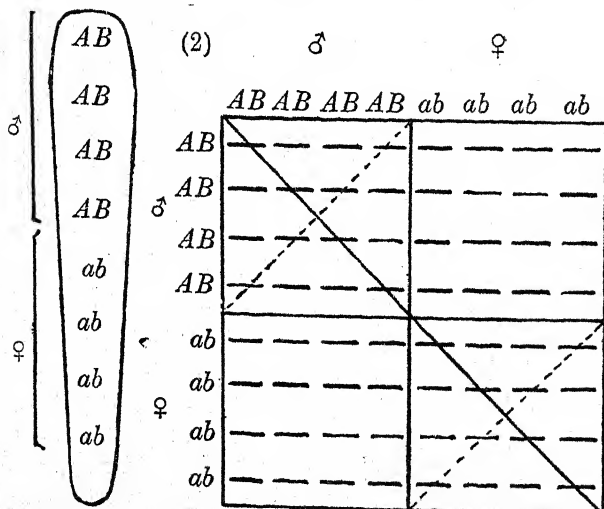
Sex and Group II segregation of *A Bab* at the
1st division.
All Group II mycelia.
32 fertile combinations possible.

SCHEME I (*continued*).

Sex segregation at the 1st division di-hybrid
bi-polar segregation of *ABab* at the 2nd division.
Mycelia fall into 2 groups 1 : 1.
No zygotes possible.

Sex and di-hybrid bi-polar segregation of *ABab*
at the 2nd division.
Mycelia fall into 2 groups 1 : 1.
16 fertile combinations possible.

SCHEME I (*continued*).

Sex segregation at the 1st division, Group I bi-
polar segregation of *A.Bab* at the 2nd division.
All Group I mycelia.
16 fertile combinations possible.

Sex segregation at the 1st division, Group II
bi-polar segregation at the 2nd division.
All Group II mycelia.
16 fertile combinations possible.

SCHEME II.

Types of segregation and the resulting reactions of the mycelia under the quadri-factorial scheme.

$$XYAb$$
$$XYAb{-}XYAb$$
(1) $XYxyABab$ ⟨   I.   Group I only, all $-$ reactions.
$$xyaB{-}xyaB$$
$$xyaB$$

$$XYAB$$
$$XYAB{-}XYAB$$
(2) $XYxyABab$ ⟨   II.   Group II only, all $-$ reactions.
$$xyab{-}xyab$$
$$xyab$$

$$XyAb$$
$$XyAb{-}XyAb$$
(3) $XYxyABab$ ⟨   III.   Group III only, all $-$ reactions.
$$xYaB{-}xYaB$$
$$xYaB$$

$$XyAB$$
$$XyAB{-}XyAB$$
(4) $XYxyABab$ ⟨   IV.   Group IV only, all $-$ reactions.
$$xYab{-}xYab$$
$$xYab$$

$$XYAb \quad \text{I.}$$
$$XYABab{-}XYaB \quad \text{I.}$$
(5) $XYxyABab$ ⟨   Groups I and II in equal numbers 1 : 1.
$$xyABab{-}xyAB \quad \text{II.}$$
$$xyab \quad \text{II.}$$
Reactions 1 + : 1 −.

$$XyAb \quad \text{III.}$$
$$XyABab{-}XyaB \quad \text{III.}$$
(6) $XYxyABab$ ⟨   Groups III and IV in equal numbers 1 : 1.
$$xYABab{-}xYAB \quad \text{IV.}$$
$$xYab \quad \text{IV.}$$
Reactions 1 + : 1 −.

$$XYAb \quad \text{I.}$$
$$XYxyABab{-}xyjaB \quad \text{I.}$$
(7) $XYxyABab$ ⟨   Groups I and IV in equal numbers 1 : 1.
$$XYxyABab{-}XyAB \quad \text{IV.}$$
$$xYab \quad \text{IV.}$$
Reactions 1 + : 1 −.

SCHEME II *(Continued)*.

$$XYAB \quad \text{II.}$$
$$XYxyABab\text{—}xyab \quad \text{II.}$$
(8) $XYxyABab$ $\Big\langle$

Groups II and III in equal numbers 1 : 1.
Reactions 1 + : 1 −.

$$XYxyABab\text{—}XyAb \quad \text{III.}$$
$$xYaB \quad \text{III.}$$

$$XYAb \quad \text{I.}$$
$$XYyABab\text{—}XyaB \quad \text{III.}$$
(9) $XYxyABab$ $\Big\langle$

Four Groups in equal numbers, 1 : 1 : 1 : 1.
Reactions 3 + : 1 −.

$$xYyABab\text{—}xYAB \quad \text{IV.}$$
$$xyab \quad \text{II.}$$

and that aversion is caused by lack of balance, then the only possible zygote of all four Groups would be the same, namely $XYxyABab$. Scheme II above shows the different types of segregation and the resulting reactions under the quadri-factorial scheme.

In the first four types of simple bi-polar segregation only one Group is possible from one and the same zygote, but a perithecium with di-hybrid bi-polar segregation in different asci can give two Groups (5) and (6).

With bi-polar segregation of one pair of factors at the first division and di-hybrid bi-polar segregation of the other pair at the second, then two Groups I and II are possible from the same zygote in (5) and two Groups III and IV in (6).

With di-hybrid bi-polar segregation of both sets of factors at the second division two Groups are again possible, I and IV in (7) and II and III in (8).

But, given that any one of the four pairs of factors can segregate at the first division and the other three at the second, then four Groups are possible from the same zygote as in (9), with a ratio of 3 + : 1 − reactions. No clear cut case of 3 + : 1 − has been found, but the very indefinite complex reactions obtained in combinations of the progeny of the dual cultures have, in some instances, given a preponderance of + over − reactions. Hence (9) cannot be ruled out.

Again, as under the bi-factorial scheme, no member of one Group can meet any member of the other Groups as in Scheme III. In this scheme only simple bi-polar segregation is allowed for and only four nuclei in each ascus are taken into account, as the third division is generally considered to be equational. There must, therefore, be at least two spores of the same genetical constitution in an ascus. If this scheme is extended to

include all possible combinations between mycelia from thirty-two asco-spores (eight belonging to each Group) the highest ratio of plus to minus reactions (not including pairs of mycelia combined with themselves along the diagonal line) would be

$$(24 \times 16) + \quad \text{to} \quad \{(8 \times 16) - 16\} -, \quad \text{or} \quad 24 : 7,$$

a ratio approximating 3 : 1.

SCHEME III.

Showing how four Groups can be obtained from a perithecium XYxyABab, and the reactions between mycelia belonging to the different Groups.

	XY Ab	XY Ab	xy aB	xy aB	XY AB	XY AB	xy ab	xy ab	Xy Ab	Xy Ab	xY aB	xY aB	Xy AB	Xy AB	xY ab	xY ab
XY Ab	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
XY Ab	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
xy aB	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
xy aB	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
XY AB	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+
XY AB	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+
xy ab	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+
xy ab	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+
Xy Ab	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+
Xy Ab	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+
xY aB	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+
xY aB	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+
Xy AB	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
Xy AB	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
xY ab	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
xY ab	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–

But neither the bi-factorial or quadri-factorial scheme explains the very general aversion found in the AG_1 and the AG_2 of SS_1, or what it is that determines the type of segregation in any given perithecium. The

ratio of $+ : -$ reactions in perithecia showing general but not complete aversion (Charts 1, 3) is considerably higher than 24 : 7, and there must be more than four biologic races carrying self-sterility factors. But there is no experimental evidence to show whether these self-sterility factors borne by the various biologic races are identical.

Discussion of the experimental results in terms of the factorial schemes.

The possible dual origin of SS_1 has already been dealt with, and the very general aversion in the first and second generations must remain unexplained.

The next point to be considered is the fertility of **113** and **111** from perithecia giving rise to such a series as XIV A (Chart 1) showing general aversion. The inocula of these cultures were single asci containing eight spores, and this would enable the mycelia from these spores to combine in all possible ways. There must have been at least four spores in each ascus of such genetical constitution as to be inter-fertile. In **113**, a Group I culture, under the four-factor scheme they would be $XYAb$ ♂ or ♀ and $xyaB$ ♂ or ♀; in **111** $XyAb$ ♂ or ♀ and $xYaB$ ♂ or ♀, being a Group III culture. On the two-factor scheme the spores of both inocula would be the same, Ab, aB or AB, ab, as far as self-sterility factors are concerned.

It would follow from this that, if it had been possible to isolate all the eight spores from either of these asci, the reactions of the mycelia would be all $-$. But seeing that the other mono-ascospore mycelia in the Series XIV A to which **113** and **111** belong, showed very general aversion, there must be some further undetermined factor or factors (possibly *inter*-racial aversion factors) over and above the four self-sterility factors, influencing the reactions due to true *intra*-perithecial aversion factors. If certain of the mycelia in **113** were carrying these undetermined factors, they would in their turn give rise to perithecia again showing very general aversion (as in Chart 3).

On the other hand, in other perithecia in the second generation, the visible segregation into mycelia belonging to two Groups (Charts 2, 2 *a*) must have been as in Scheme II (5), bi-polar for $XYxy$ at the first division and di-hybrid bi-polar for $ABab$ at the second. Sex also must have segregated at the second, seeing that another generation has been raised from this series (Charts 4, 5, 6). If sex had segregated at the first division no zygote would have been possible (cf. Scheme I (3)).

The appearance of Chart 2 at first sight suggests that the reactions might be due to simple segregation of sex, mycelia of the same sex

showing aversion. But this is not the case. In the next generation, among the progeny of **203** showing no intra-perithecial aversion, some of the dual combinations proved fertile, and therefore mycelia of both sexes must have been present in Series XXXV and XXXVI (Chart 4).

No definite conclusion can be arrived at from the results of the AG_2 as to whether it is possible for a Group I culture to produce the three kinds of perithecia on p. 19, as the mycelia of Series XIX A and XIX B are too few in number. But it is theoretically possible, and experimental proof is forthcoming from the results of the AG_4, where two Group I mono-ascospore mycelia in dual culture have given perithecia showing no *intra*-perithecial aversion (Chart 8).

To pass on to the next generation; in Chart 4, giving the AG_3 of SS_1 derived from perithecia in mono-ascus culture **203** (Group II segregate from Group I **113**, Charts 2, 2 a) all the mycelia meet, all give Group II reactions to Group I cultures and all meet the parent culture. Here the segregation of self-sterility factors would be as in Scheme II (2), but if sex had segregated at the first division, thirty-two fertile combinations would be expected. The number of fertile dual cultures was, however, surprisingly low (at the most four, but probably only two out of thirteen), and hence sex segregation is more likely to have occurred at the second division (cf. Scheme I (6)), with only sixteen possible fertile combinations out of sixty-four.

On the other hand, in Charts 5 and 5 a, giving a series derived from another AG_3 mono-ascus culture **211** (again a Group II segregate from the same Group I culture **113**), the mycelia show visible segregation and fall into the same two Groups, I and II, in the ratio of eighteen Group I to thirteen Group II. The numbers are small as compared with the large number of spores present in a single perithecium, and this ratio may be taken as approximating equality, more especially as the series was derived from possibly more than one perithecium.

211 being a Group II culture, the segregation in the ascus used as inoculum would be bi-polar for $XYxy$ (XY, xy) and $ABab$ (AB, ab) at the first division; but in the asci giving rise to the next generation in the perithecia formed by **211**, the bi-polar segregation of $XYxy$ would be the same at the first division, and quadri-polar for $ABab$ (AB, Ab, aB, ab) at the second, either di-hybrid bi-polar in different asci or quadri-polar in the same ascus. As fertile progeny have been obtained from this series (Table A) sex segregation must have taken place at the second division (Scheme I (4)). The parent culture meets all Group II mycelia and shows aversion to the Group I mycelia.

It will be seen in Scheme I (4) that, out of thirty-two combinations showing no aversion half the number (sixteen) would be fertile, and this would account for the higher percentage of fertile dual cultures from **211** (Table A 18 : 9), as compared to those from **203** (Table B 12 : 4, or 12 : 2).

Further, in Chart 6, Group I mono-ascus culture **205** and Group II mono-ascus culture **206** (also in the AG_2 of SS_1), although they belong to different Groups, have each in their turn given two different types of segregation, as did their two sister cultures **211** and **203** respectively. Moreover **205** and **206** were mono-ascus cultures from the same perithecium. The segregation in the perithecia of **205** would be bi-polar for $XYxy$ (XY, xy) and quadri-polar for $ABab$, and in **206** bi-polar for both sets of factors.

Throughout the investigation mono-ascus cultures (with the exception of the sectoring cultures in Charts 2, 2 a) have always behaved just as any of the other mono-ascospore cultures belonging to the same Group, although these experiments have made it quite obvious that the spores of an ascus are not all genetically the same. It must be borne in mind that a mono-ascus culture is only an aggregate of at the most eight haplo-mycelia, and that although there must be an interchange of nuclei at some period if the cultures prove fertile, this interchange cannot influence the genetical constitution of the mycelia themselves until fusion of nuclei has taken place immediately preceding or during the development of the asci in the perithecia borne on the mycelia. The only explanation for the consistent behaviour of mono-ascus cultures is that quadri-polar segregation has not occurred in the inoculum ascus. Thus, all the haplont mycelia in the mono-ascus culture **113** would be $XYAb$, $xyAb$, $XYaB$, $xyaB$; and in the same way, the haplont mycelia in **203** and **211** would be $XYAB$, $xyAB$, $XYab$, $xyab$. These cultures would, therefore, meet other mono-ascospore cultures belonging to the same Group, although they are capable of showing different types of segregation in their progeny.

With regard to the two sectoring cultures **205** and **206**, if the sectoring was due to the presence in the inocula of spores of different genetical constitution such as to cause aversion between the mycelia, as shown by the sector being cut off from the parent colony by a clear line of demarcation, then quadri-polar segregation must have occurred in the inoculum ascus. Theoretically these cultures should not behave consistently as a whole towards other mono-ascospore mycelia belonging to the same Group. This was found to be the case before sectoring was detected (*vide*

pp. 13, 14), but by subculturing the parent colony after it had thrown the sector, the sectoring mycelia were eliminated.

The parent culture **205** was derived from a sub-culture of the original stock while it was comparatively young, and before it had begun to sector. This culture developed the perithecial stage very slowly, and it was not till nearly two years after that the perithecia giving rise to the next generation (Series XLIII, Chart 6) were dissected out. The sub-culture of **205** used to test the progeny had necessarily gone through many transfers during that period and had behaved consistently for some time as Group I, the mycelia of Group II having been eliminated. The same process of elimination was adopted for **206** (*d*) but in this case it was the purified culture which produced perithecia giving rise to Series XLII, Chart 6.

The parallel series derived from mono-ascus culture **111** (sister-culture to **113**) must now be considered.

To return to the AG_3, in Charts 7 and 7 *a* the progeny of **111** also show visible segregation into two Groups, III and IV, one giving the reverse reactions to the other, and the parent belongs to one of the Groups, viz. Group III. But when the two Groups from **113** were tested with III and IV they both showed aversion irrespective of group.

The dual cultures of the mono-ascospore mycelia from **111** showing no mutual aversion have all proved absolutely sterile. Every effort was made to try and induce the production of perithecia but without success. This again was very surprising, seeing that so many fertile cultures were obtainable from **211** (Table A), which also gave visible segregation into two Groups. This complete sterility can be explained by the type of segregation set out in Scheme I (3), sex and bi-polar segregation of $XYxy$ at the first division and di-hybrid bi-polar segregation of $ABab$ at the second. The mycelia fall into two Groups, but all those which meet are of the same sex.

On the quadri-polar scheme, if the segregation of $XYxy$ for Groups I and II is XY, xy, and for Groups III and IV, Xy, xY, then neither of the Groups I and II will meet either of the Groups III and IV; hence the two Groups from **113** cannot meet either of the Groups from **111**.

Groups I and II have the same bi-polar segregation of $XYxy$ giving homo-haplonts XY, xy, but differ as to the segregation of $ABab$, and Groups III and IV also have bi-polar segregation, but giving rise to hetero-haplonts Xy, xY, and differ in the same way as Groups I and II as to the segregation of $ABab$. In both cases the segregation of $XYxy$ is bi-polar, but $ABab$ may be either bi-polar or quadri-polar, and therefore

any Group can throw the opposite Group in its progeny if quadri-polar segregation of *ABab* occurs.

There is the possibility of quadri-polar segregation of *XYxy* also, in which case one Group could throw all three other Groups from the same perithecium. The ratio of + : − reactions would be the same as in Scheme III (1) 24 +$_e$: 7 −.

Thus all the Groups have zygotes of the same genetical constitution but the Groups differ in pairs as to the bi-polar segregation of *XYxy*; they may all give quadri-polar segregation of *ABab*, but each individual Group has its own type of bi-polar segregation.

The chief difficulty experienced in recording the reactions of the mycelia derived from dual cultures was that a line of demarcation showed between the colonies when they first met, but was not permanent. In some combinations the line disappeared entirely in the course of a few days, in others the line persisted but was not well defined, and again in others it persisted but was never so pronounced as in previous generations. Whether these varying degrees of aversion were due in some cases to weakened mycelia, and in others to some modified form of aversion not previously met with, cannot be determined. Mycelia showing no aversion always intermingled quite freely from the first. If the doubtful combinations were recorded several days in succession the early records differed entirely from the later ones. The weakened mycelia gradually lose their capacity of forming pycnidia and pycnospores, and several stock cultures have died out. Hence, it was not possible to interpret the results satisfactorily, but a few generalisations can be made. The reactions between mycelia from perithecia showing these varying degrees of aversion appear to be quite promiscuous and cannot be sorted into definite Groups.

A considerable number of perithecia from different dual cultures of Group I segregates from Group II culture **211** (Table A) have been partially tested, and the results from different cultures are extremely variable, although all the dual cultures in each table have one parent in common. Some dual cultures from **211** gave perithecia showing no definite *intra*-perithecial aversion, as in Chart 8 (Group I mycelia, *602* and *618*). The − ? sign indicates that although the colonies met, a slight but persistent ridge showed along the line of contact and the intermingling was not quite free. The general appearance of the chart, however, indicates no true *intra*-perithecial aversion and all the mycelia show aversion to Group II cultures but meet the Group I parent cultures.

But it will be seen that the mycelia mostly show aversion towards **113**, itself a Group I culture. The + ? indicates that the line of aversion

was rather indefinite. Four perithecia from this dual culture were tested and all gave similar results.

CHART 8.

Series XLVIII. MP.

746 −47 −48 −49 −50 −51 −52 −53 −54 −55 −56 −57 −58 −59 −60 −61 −62 −63 −64 −65

Group II	203	+ + + + + + + + + +	
Group II	211	+ + + + + + + +	
Group I	602	— — — — — — — —	
Group I	618	— ? — — — — — —? — — ● — — —	
Group I	113	+ + + + + +? + + + + + +? + + +	
SS₁		+ + + + + + + + + + + + +	
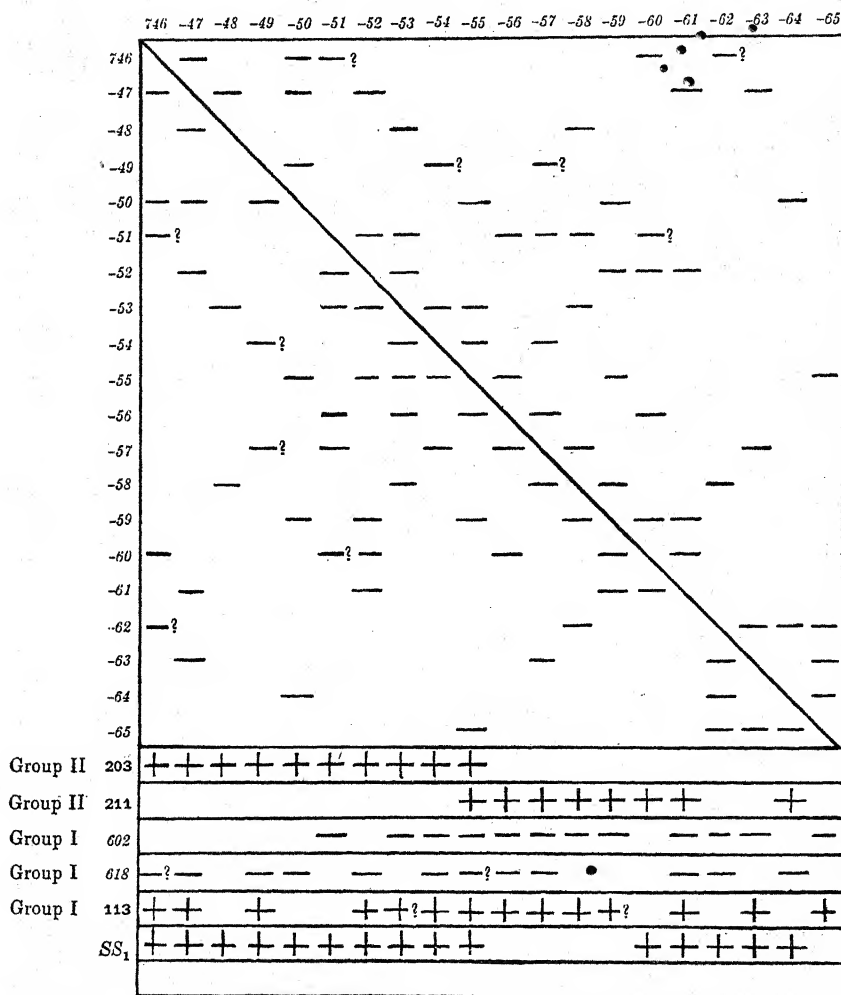
AG_4. Dual culture CA 9 (Table A). Progeny of **211**.
Group I haplonts combined Nos. *602* and *618* (see Chart 5 *a*).

CA 8 being a culture of two Group I mycelia, and showing no *intra*-perithecial aversion, the segregation of the aversion factors must have

been bi-polar in both cases, *i.e. XY, xy, Ab, aB.* But why all the mycelia show aversion to **113** (itself a Group I culture) cannot be explained satisfactorily on the data to hand. It may be that since SS_1 and **113** have both shown that they are capable of throwing more than two Groups in their progeny, the presence of mycelia other than Groups I and II may cause this general aversion to all the mycelia tabulated in Chart 8, more especially as **113** had been growing in culture for some years when these tests were made, and possibly the ascospores in various perithecia had germinated *in situ*, and that the culture was more complex in constitution than in the earlier stages of the investigation.

Again, other dual cultures of the same series (Table A) have given perithecia showing variable degrees of aversion (indicated in Table D by the signs, ⧺ +, ±, −) and very irregular segregation. No chart is given showing a series with variable degrees of aversion, as the records are not reliable for the reasons given above, and it is not possible to group the results in any kind of order.

Further, other Group I dual cultures from **211** have given perithecia showing a preponderance of − over + reactions.

The only series of cultures (Series LVIII, Chart 9) obtainable from dual cultures of the Group II progeny of **203** has not shown this variability, but the mycelia again fall into two Groups giving reverse reactions, and both parents belong to one Group and show aversion towards mycelia of the opposite Group.

These results show that **203** was not pure for no aversion.

In this chart there is considerable excess of one Group over the other 18 : 8, but here again the number of cultures is small and the ratio may represent equality. The mycelia belonging to both Groups show aversion to **113**, and further, the mycelia which meet the immediate Group II parents *576* and *584* show the reverse reactions to **203** and **211** Group II stock cultures. Now the parents *576* and *584* themselves showed Group II reactions (*vide* Chart 4) and met **203** and **205** (*d*), and this second case of complete reversal must also remain unexplained. The mycelia in this Series LVIII, however, showed differences in growth habit correlated with the Group to which they belonged, and there may be some other factor coming into play. The mycelia belonging to the upper Group in Chart 9, *977–997*, showed whitish colonies with normal pycnidial development, the lower numbers, *973–998*, greyish colonies with very few pycnidia. The differences in habit in this series was not investigated further, as considerable difficulty was experienced in keeping the grey cultures growing normally. Also, it can be seen that the reactions between the

mycelia of this series and the stock cultures have been very indefinite in some cases.

CHART 9.

Series LVIII. SP.

(Chart: grid of + and − symbols. Top axis labels: 973 −74 −75 −76 −81 −82 −83 −85 −86 −87 −88 −90 −91 −93 −94 −95 −96 −98 −77 −78 −79 −80 −84 −89 −92 −97)

Upper chart row labels: 977, −78, −79, −80, −84, −89, −92, −97; Group II 203; Group II 211; Group I 113; SS₁.

Lower chart row labels: 973, −74, −75, −76, −81, −82, −83, −85, −86, −87, −88, −90, −91, −93, −94, −95, −96, −98; Group I Parents { 576, 584 }; Group II 601.
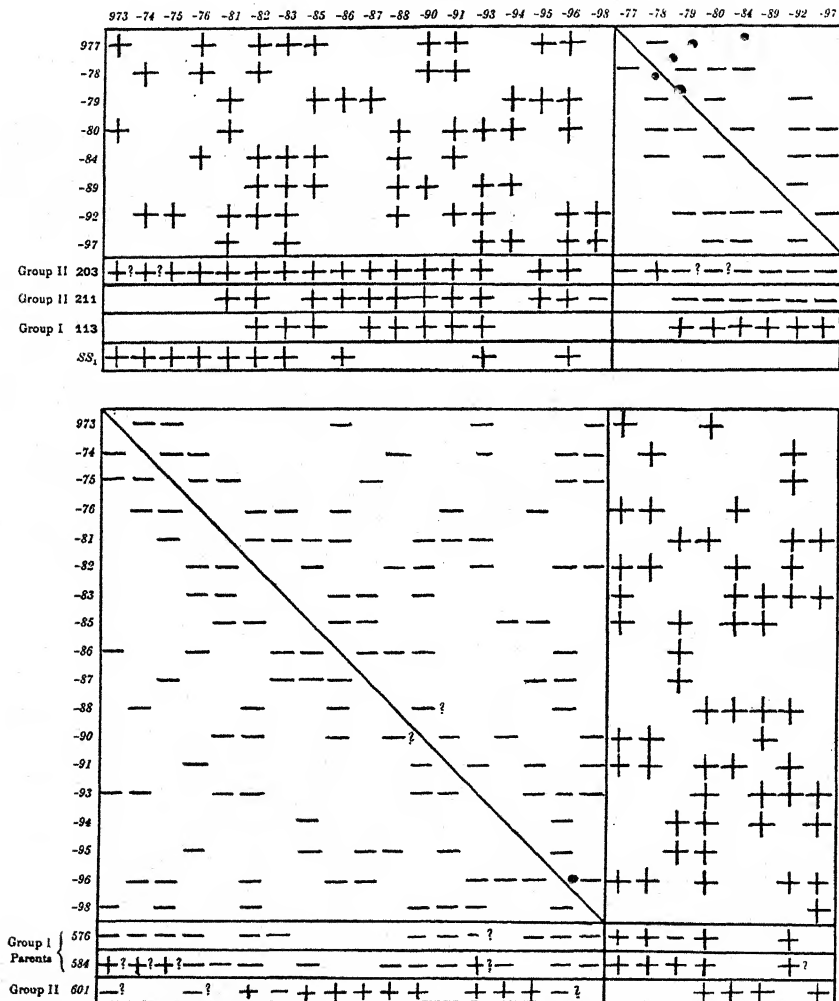
AG_4. Dual culture CA 25 (Table B). Progeny of **203**.
Group II haplonts combined Nos. *576* and *584* (see Chart 4).

In Table C comprising dual cultures of the progeny of another Group II culture, **206**, perithecia have again occurred showing the same variable

degrees of aversion, as found in the Group I dual cultures of Table A, although Group II in previous generations had not shown itself capable of throwing more than two Groups. In these dual cultures, also, differences in growth-habit was found.

Unfortunately, owing to the long life cycle of this fungus, no kind of interpretation of the experimental results could be arrived at until a large mass of data had been accumulated extending over several years, so that many important points were not realised during the course of the work until the cultures were too old or had been discarded. Hence this discussion leaves much to be desired, and the explanation of the various points brought out by these experiments is only put forward tentatively for lack of conclusive evidence.

The results obtained when the cultures were young and vigorous are fairly consistent and clear cut, so that some, at least, of the later conflicting results may be attributed, in part, to deterioration or change of constitution in cultures of long standing.

Although the problem remains still very complex, there is good evidence to show that the factors bringing about aversion between the colonies are heritable and segregate on Mendelian lines. The results obtained from dual cultures derived from **203**, **211**, and **111** show fairly clearly that the self-sterility factors can segregate independently of sex, and hence are not positive sex factors. The most striking points are, however, that as in the Basidiomycetes, different types of segregation can occur in different perithecia in the same culture, and in the progeny from different asci in the same perithecium; that two Groups, or possibly more than two, can be obtained from the same perithecium, and that although the mycelia show inter-groupal aversion, they can throw another Group in their progeny.

These facts can be satisfactorily explained up to a certain point by a bi-factorial scheme of inheritance, but the complex results of the AG_4 suggest that there are more than two pairs of self-sterility factors.

In *D. perniciosa* the results, as far as they go, indicate that two forms of sterility (*inter*-racial and self-sterility) influence the sexual reactions between mono-spore mycelia in a haplo-heteroecious fungus.

The multicellular multinucleate archegonium described in a previous paper (Cayley, 1923 *b*) from which the perithecium arises may be looked upon as a female organ, but the cells of this archegonium disintegrate during the development of the perithecium. In a mono-spore culture this female organ can produce the outer structure of the perithecium but no asci. Thus the essential nuclear fusions must take place in the perithecium

itself, and not previously. The sterile perithecia-like structures have been found in some mono-ascospore cultures but not in all, and those which do not produce these structures are possibly male mycelia. Sterile perithecia have been found in dual cultures also, in some cases this may be due to the combination of two female mycelia, in others to the combination of mycelia of such genetical constitutions that they can meet but cannot fuse (e.g. $XYAB$ ♂ and $XYAB$ ♀, etc.). Nothing is known as to when the interchange of nuclei of different sexes occurs after the two mycelia have met, whether immediately before the development of the perithecium, or shortly after the colonies have come in contact.

Different forms of heterothallism in the Fungi.

Within recent years, the many valuable additions to our knowledge of heterothallism in its various forms call for a more precise terminology in order to avoid confusion between true sex heterothallism and the effect produced by heritable factors other than sex on the sexual affinity of the haplont mycelia. An attempt is made below to classify these forms.

1. Simple haplo-heteroecism (true sex heterothallism) as found in the Phycomycetes, Uredinieae, Ustilaginieae (with the exception perhaps of *U. grandis*, and the forms deviating from the normal in *U. longissima* found in some localities (Bauch, 1923; Kämmerling, 1929), in the Ascomycetes such as *Neurospora, Ascobolus, Penicillium*, etc.

2. Haplo-heteroecism together with physiological heterothallism other than sex based on *inter*-racial and self-sterility factors influencing the sexual affinity of the haplonts, but segregating independently of sex, as in *D. perniciosa*.

3. Haplo-heteroecism together with other forms of physiological or morphological heterothallism not influencing the sexual affinity of the haplonts but segregating independently of sex, as in *Neurospora sitophila, Schizophyllum commune, Coprinus Friesii* and *C. ephemerus* (Brunswik, 1924 c), and *Collybia velutipes* (Zattler, 1924). In *Collybia velutipes*, however, combinations of recessive albino forms produced no fruiting bodies.

Suppositional.

4. Heterothallism based on self-sterility factor or factors in a haplo-synoecious fungus, as in *Humaria granulata* and possibly the heterothallic Hymenomycetes.

5. Heterothallism based on *inter*-racial sterility factors in a haplo-synoecious fungus carrying self-sterility factors also, as in *Coprinus micaceus*.

E D.

Genealogical table of ascospore generations of SS_1.

PG_1 - - - - - - -

AG_1 - - - - - - - I-IV
$+ + + + +$ Chart 1

] Group I   111 Group III

AG_2 - - - - - $+ + + + + +$ Chart 3   Group II Group I $- - - -$ Charts 2, 2a   Group III Group IV $- - - + + + +$ Charts 7, 7a

203   211   205   Dual Cultures Sterile

Chart 6

AG_3 - - - Group II $- - - -$ Chart 4   Group II Group $- - - + +$ Charts 5, 5a   Group II $- - - -$ Chart 6   Group I Group II $- - - + + + +$
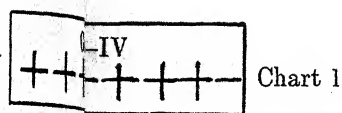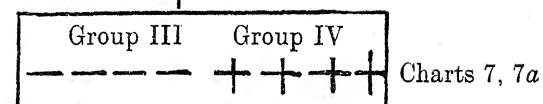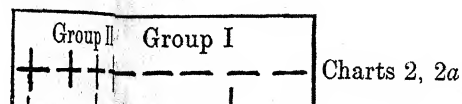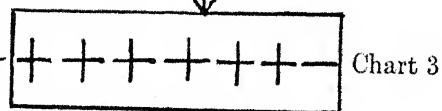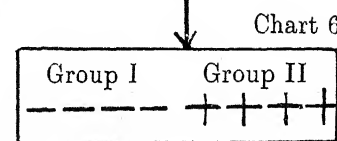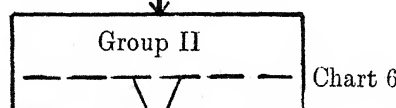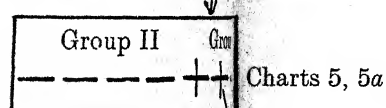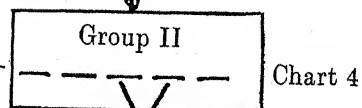
Dual Group II Cultures *576* and *584*   Dual Group Cultures   Dual Group II Cultures

AG_4 - Group I Group II $- - - + + + +$ Chart 8 $- - - -$ $+ + + - -$ $+ + - -$ $++ ++ + + + - - -$
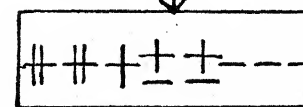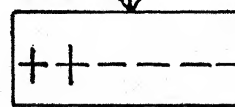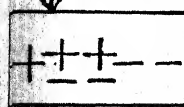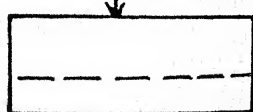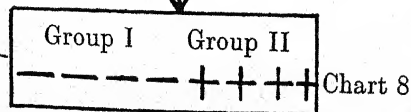
SUMMARY.

D. perniciosa has proved to be both haplo-heteroecious and hetero-thallic for the capacity for showing mutual aversion between mono-spore mycelia, and this capacity is inherited and segregates in subsequent generations.

There are two forms of aversion, *inter*-racial, shown by both the haplo-synoecious and haplo-heteroecious forms of *Diaporthe* found asso-ciated with "die-back" in fruit trees, and *intra*-perithecial aversion shown by the haplo-heteroecious form only.

Inter-racial aversion is a form of sterility between biologic races; *intra*-perithecial aversion is a peculiar form of physiologic self-sterility other than sex.

The reactions produced by these two forms of aversion are indis-tinguishable to the naked eye.

Sex does not influence the manifestation of mutual aversion between mycelia.

Intra-perithecial aversion occurs between mycelia carrying certain given combinations of self-sterility factors. These factors segregate in-dependently of one another and of sex, giving rise to two or four distinct Groups of mycelia.

The zygote nucleus in the ascus of all four Groups is of the same genetical constitution, but the Groups differ as to the combinations of the factors present in the haploid mycelia.

The segregation of the self-sterility factors may be either bi-polar, or di-hybrid bi-polar (quadri-polar) in the same ascus or in different asci of the same perithecium.

Mycelia belonging to one Group show aversion towards all the mycelia belonging to any other Group, but on account of the segregation of the *intra*-perithecial aversion factors, one Group can throw mycelia of another Group in its progeny.

Four ascospore generations have been raised and tested from the haplo-heteroecious pycnospore culture SS_1, and the results discussed in terms of a factorial scheme.

The occurrence of aversion or no aversion among the progeny of a

single perithecium depends upon the type of segregation that has occurred in the various zygote nuclei of the asci in the parent perithecium.

Different asci in the same perithecium can give different types of segregation of the self-sterility factors in their progeny.

The occurrence of aversion in other genera of fungi and the correlation of aversion with sex is discussed.

A satisfactory interpretation of the complex results during the latter part of the investigation has not been possible, partly owing to the weakened condition of the cultures, due either to prolonged growth on artificial media or to close in-breeding.

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EXPLANATION OF PLATE I.

Various test platings for both forms of aversion, *inter*-racial and *intra*-perithecial, on oatmeal agar.

Synoecious form of *Diaporthe*.

Fig. 1. Two mono-ascospore cultures from different perithecia on the same host. 11 days old. *No aversion*.

Fig. 2. (1) Mono-ascus culture from peach, (2) mono-ascospore culture from plum. 11 days old. Inter-*racial aversion*.

Heteroecious form of *Diaporthe*.

Fig. 3. (1) Mono-pycnospore culture from plum var. unknown, (2) mono-ascospore culture from seedling plum, No. 195. 8 days old. Inter-*racial aversion*.

Fig. 4. Three mono-ascospore AG_4 cultures from the same perithecium, progeny from dual culture CA 52 (Table C). 4 days old. *No intra-perithecial aversion*.

Fig. 5. AG_2 mono-ascus culture 205 (Charts 2, 2 *a*) throwing sector (*d*). 5 days old.

Fig. 6. 205 tested with its sector (*d*). 5 days old. Intra-*perithecial aversion*.

Fig. 7. The original plate containing SS_1 and two other mono-pycnospore isolations from different pycnidia. 7 days old. *Probably both* inter-*racial and* intra-*perithecial aversion*.
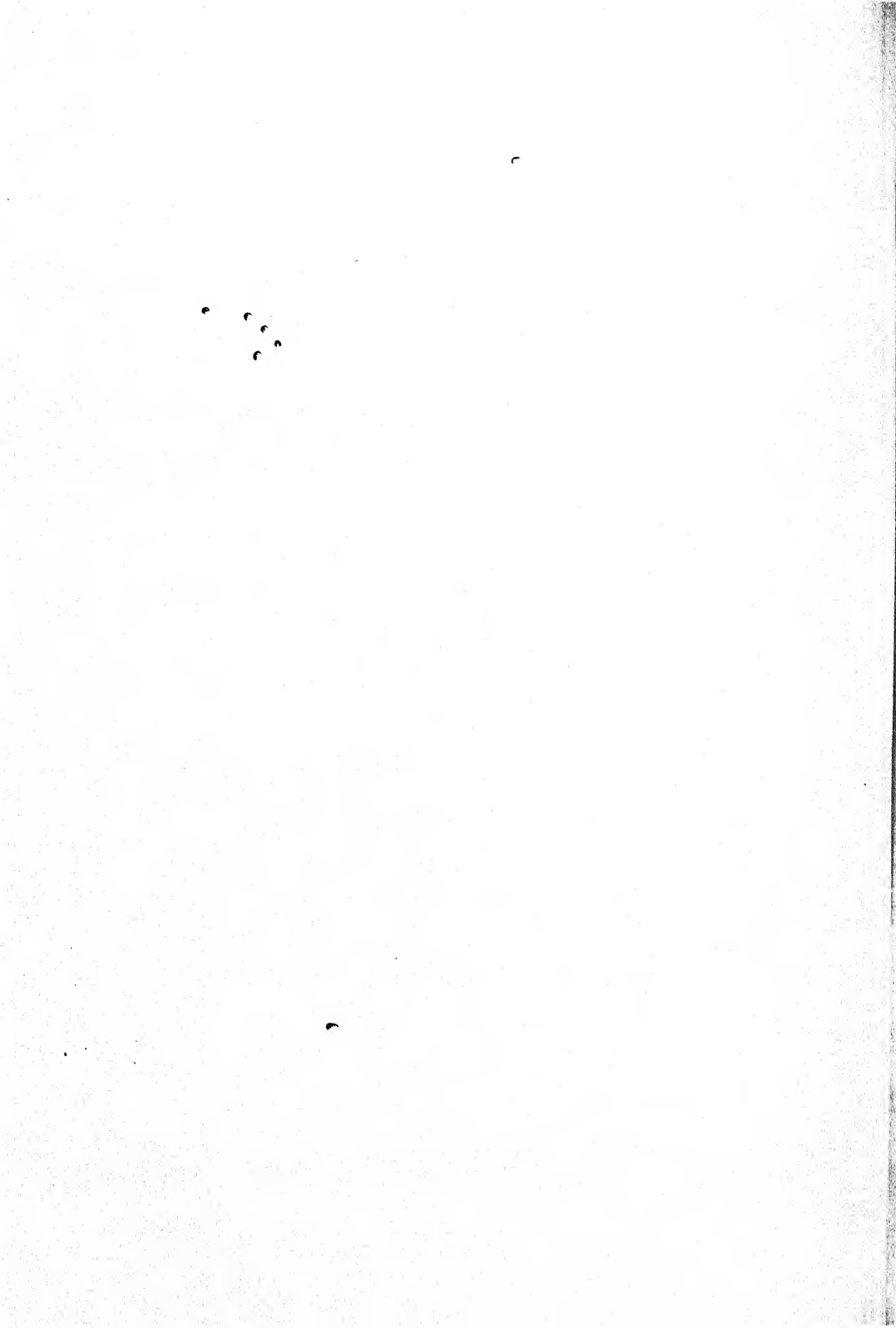
Fig. 8. The same cultures re-tested after 7 months. 9 days old.

Fig. 9. Three mono-ascospore cultures: (1) and (2) from the same perithecium, (3) from another perithecium on the same host. 16 days old. *Probably* intra-*perithecial aversion*.

Synoecious and heteroecious forms of *Diaporthe* combined.

Fig. 10. Three mono-ascospore cultures: (1) and (2) synoecious, (3) heteroecious forms. 7 days old. Inter-*racial aversion*.

Heteroecious form.

Fig. 11. Four mycelia: (1) SS_1, (2) Group I AG_2 mono-ascus culture **205**, and two Group II sector cultures thrown by **205** (*205 (b)* (3) and *205 (d)* (4)). 9 days old. Inter-*racial and* intra-*perithecial aversion between* SS_1 *and the other cultures;* intra-*perithecial aversion between* (2), (3) and (4).

Fig. 12. Mono-ascospore sowings from one and the same perithecium in SS_1. 6 days old. *Very general aversion*.

Fig. 13. SS_1 and AG_2 mono-ascus Group II culture **211**. 6 days old. *Probably both* inter-*racial and* intra-*perithecial aversion*.

Fig. 14. SS_1 and **211** and Group I progeny of **211**, culture *602* (Charts 5, 5 *a*). 6 days old. Inter-*racial and* intra-*perithecial aversion between* SS_1 *and* **211**. Intra-*perithecial aversion between* **211** *and* 602.

Fig. 15. **211** tested with itself. 7 days old. *No aversion*.

MEIOSIS IN DIPLOID AND TETRAPLOID
PRIMULA SINENSIS.

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(John Innes Horticultural Institution, Merton.)

(With Twenty Text-figures and Three Diagrams.)

CONTENTS.

I. INTRODUCTION.

CHROMOSOME behaviour in *Primula sinensis*[1] calls for study on two grounds. First, linkage phenomena in this species have been investigated in some respects more thoroughly than in any other plant. In no organism whose linkage properties have been determined have the relevant stages of meiosis also been described[2]. This is an objection (perhaps not too serious) to the theories of crossing-over now current. Secondly, the giant *Primula sinensis* is a tetraploid of known origin and exactly comparable with the diploid. Every hereditary element present in the diploid is, we believe, represented four times in the tetraploid. A comparison of its meiosis will, therefore, show those differences in chromosome behaviour which are to be associated with the change in chromosome number, and not, for example, with hybridity. Such differences therefore afford a means of testing the various hypotheses implicit in the chiasma theory

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[1] The history of this "species" is described by Gregory, de Winton and Bateson (1923).

[2] Chromosome numbers have been counted at meiosis in normal and giant *Primula sinensis* by Gregory (1909, 1914) and at somatic mitosis in the normal by Vokolek (1925). Belling and Blakeslee (1924) and Belling (1927) have mentioned the occurrence of quadrivalents at meiosis in the tetraploid giant.

Cytological observations on *Drosophila* are referred to in Section VIII.

of pairing. It will also show whether any modification of the straight-forward assumptions made by Blakeslee and his collaborators in *Datura* may be necessary in this case.

P. sinensis, like other species of *Primula*, is not in most respects favourable material for detailed cytological study. Preparations that were particularly satisfactory for diakinesis and metaphase were, how-ever, obtained with a modification of La-Cour's first fixative (1929) in which the sodium sulphate and urea were replaced by 0·05 gm. of saponin.

Smear methods proved useless. Material was cut at 16 to 20 μ and stained with gentian violet (cf. Newton and Darlington, 1929). The amount of material available from one plant was too small to make the study of single plants practicable. Nor was it possible to ascribe signi-ficant differences in observation to difference of family. I am indebted to Miss de Winton for abundant material of strains of diploid and tetra-ploid *Primula sinensis*, inbred for from ten to fifteen generations.

II. Somatic chromosomes.

The somatic numbers of typical and giant *Primula sinensis* are 24 (Fig. 1) and 48 (Fig. 2) respectively. One or two types can be picked out definitely as occurring twice in the diploid and four times in the tetraploid. That marked *a* is the only chromosome with a subterminal constriction. This is of importance in considering the types of con-
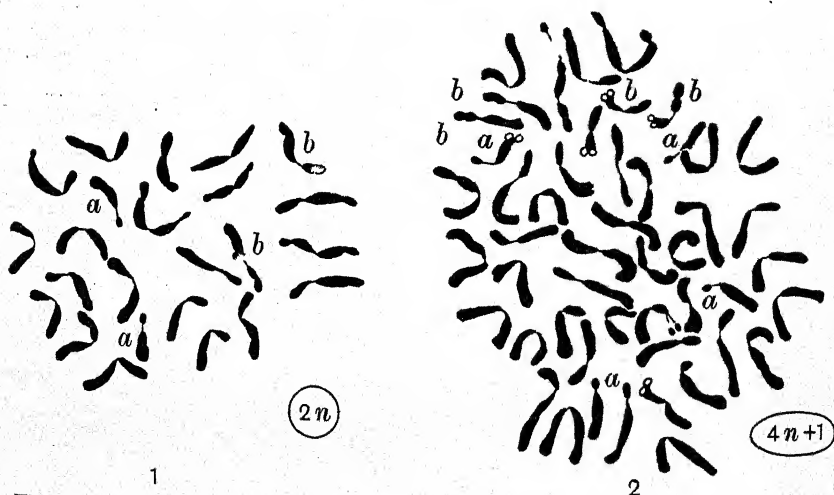
Figs. 1 and 2. Somatic mitosis in diploid and approximately tetraploid ($4n+1$) *Primula sinensis*; corresponding types lettered. (× 5700.)

figuration at meiosis. The chromosomes at metaphase in a somatic mitosis are 1·5 to 3 μ long. They are larger than in *Primula kewensis* (Newton and Pellew, 1929) or (Mr Philp tells me) in *P. obconica*. The shortest are the length of the X chromosome; the longest, the length of the second and third chromosomes in *Drosophila melanogaster* (cf. Stern, 1929 *a*, Fig. 1).

Here, as in comparable cases that I have noticed, the equatorial plate in the tetraploid cells is intermediate in area between being (i) twice that of the diploid at the same stage, as comparable spacing requires, and (ii) $2^{\frac{2}{3}}$ or 1·6 times that of the diploid, as doubling in cell-volume and spindle-volume requires. The chromosomes are therefore as a rule more crowded in the tetraploid than in the diploid.

My colleague, Mr Philp, and others have determined the chromosome number in fifty-three of the following cases taken from Miss de Winton's experiments:

Cross	Nature of cross	Family No.		Result
$1^2/22 \times 7^3/22$	$4n \times 2n$	39/23	(2 plants)	ca. $3n$
$135^3/25 \times 103^2/25$	,,	144/26	(1 plant)	ca. $3n$
$141^2/26 \times 33^1/26$	,,	131/27	,,	$3n$
$142^2/26 \times 7^1/26$	,,	132/27	,,	$3n$
$155^1/27 \times 13^2/27$	,,	205/28	,,	$4n+1$
$255^3/27 \times 93^1/27$	,,	206/28	,,	ca. $4n$
$144^1/26 \times 116^1/26$	$3n \times 2n$	135/27	,,	$2n+2$
135/27 selfed	$(2n+2) \times (2n+2)$	210/28	(6 plants)	$\begin{cases} 2n \\ 2n+1 \end{cases}$
$147^2/27 \times 135/27$	$4n \times (2n+2)$	212/28	Nil	—
$12^3/27 \times 135/27$	$2n \times (2n+2)$	214/28	,,	—
$11^4/27 \times 135/27$	,,	213/28	(1 plant)	$2n$
$75^2/27 \times 135/27$	,,	215/28	(8 plants)	?
			(32 plants)	$2n$
			(Part of 1 plant)	$4n$
$140^1/26 \times 144^1/26$	$4n \times 3n$	138/27	(1 plant)	$?4n-1$
138/27 selfed	$(4n-1) \times (4n-1)$	216/28	(2 plants)	$\begin{cases} 4n-1 \\ ?4n+3 \end{cases}$
$141^1/27 \times 138/27$	$4n \times (4n-1)$	217/28	,,	$4n$ (1)
$141^2/27 \times 138/27$	,,	218/28	,,	$?4n$ (1)
$150^1/27 \times 138/27$	,,	219/28	,,	$4n-1$
$266^2/27 \times 138/27$	,,	220/28	(5 plants)	$?4n-1$ (1)

TABLE I.

Primula sinensis in 1929. Families taken as examples in a favourable year. Showing the fertility of diploid and tetraploid.

	No. of capsules fertilised	Failures	Capsules set	Seeds	Average no. of seeds	Percentage germination
Diploid types selfed	44	12	32	376	11·75	72·3
Diploid linkage back-cross	30	—	30	965	32·2	80·7
Tetraploid types selfed	44	21	23	152	6·6	46
Tetraploid linkage back-cross	29	1	28	652	23·2	77·7

TABLE II.

Showing the fertility of crosses in Primula sinensis *in 1926 and 1927.*

Cross	Capsules pollinated	Failures	Capsules set	Seeds	Result
Diploid × tetraploid	103	102	1	1	No germination
Tetraploid × diploid	109	103	6	6	2 failed
					2 triploid
					2 tetraploid
Triploids selfed	41	41	—	—	—
Triploid × diploid	17	15	2	2	1 failed
					1, $2n=26$
Diploid × triploid	40	36	4	6	No germination
Triploid × tetraploid	17	16	1	4	No germination
Tetraploid × triploid	36	30	6	24	3 germinated:
					1, $2n=52$
					2, $2n=47$

The important facts to be derived from these and earlier observations are the following:

(i) Somatic doubling can occur in the diploid *Primula sinensis*.

(ii) Haploid pollen grains produced by the diploid are functional in fertilising the tetraploid.

(iii) Functional diploid pollen grains are also produced by the diploid.

(iv) The tetraploid will not cross on to the diploid (cf. *Datura Stramonium*).

(v) The five triploid seedlings produced were highly sterile.

(vi) Inbred races of both diploid and tetraploid are less fertile than outbred races.

(vii) The tetraploid, like all other auto-tetraploids, is less fertile in every stage of development than the diploid which has given rise to it (cf. Darlington, 1928).

(viii) The tetraploid has arisen from the diploid several times. The absence of spontaneous triploids (though the possibility of their occurrence cannot be excluded) points, as in *Datura*, to a somatic origin.

III. Meiosis in the Diploid.

There is a normal pachytene stage following the association of the chromosomes in pairs side by side. This is followed by diplotene. At this stage it is not possible to study the whole complement, but isolated observations of bivalents (Fig. 3) show that chiasmata (where the chromatids, or half-chromosomes, now associated in pairs, change partners) are formed at random, or at least without evident interference with one another. An interference corresponding to the genetical observations could be determined only by more detailed analysis than I have been able to make. They vary in number from two to five for each

bivalent. The lower number may be an under-estimate, owing to such bivalents having been cut.

Between this stage and diakinesis two orderly changes can be seen. First, there is a reduction in the number of chiasmata; secondly, an increasing number of bivalents have terminal chiasmata. Evidently the change is of the same kind as that illustrated for single chiasmata by Wenrich (1916). This movement of chiasmata, which I have called terminalisation, is probably more correctly described as a movement *away from the point of attachment,* for the association of the chiasmata
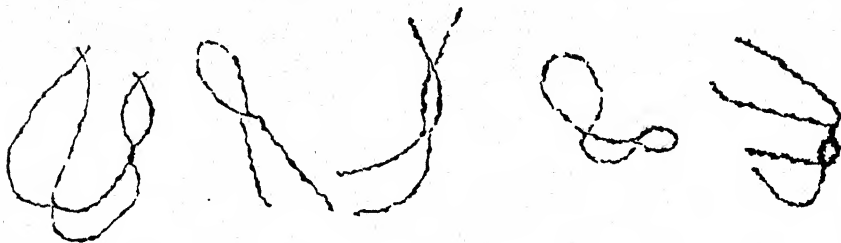
Fig. 3. Diplotene in the diploid. Numbers of chiasmata given with each bivalent. (× 5700.)

with the attachment in *Fritillaria Meleagris* (Newton and Darlington, 1930) shows that it has to be reckoned with at this stage. On this assumption a chromosome with a subterminal attachment (type *a*, Fig. 1) would more rarely give a ring at diakinesis than one with a median attachment. It is possible that one chromosome pair forms a rod with particularly high frequency.

The intermediate stages in terminalisation illustrated (Fig. 4) are to be found in the same nucleus, some bivalents having chiasmata already terminal, no doubt owing to these having been originally formed near the end. The absence of any crowding of chiasmata near the ends shows
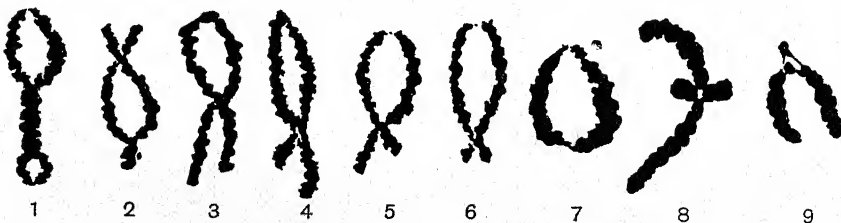
Fig. 4. Stages between diplotene and diakinesis in the diploid, showing the process of terminalisation. The two rightmost figures have chiasmata (according to the assumption made) only on one side of the attachment constriction and will give rods with one chiasma. The others have chiasmata (two to four) on either side of the attachment and will give rings with two terminal chiasmata. (× 5700.)

that the chiasmata which are formed near the ends move as early and as quickly towards the ends as those formed nearer the attachment constriction.

Owing to this movement the condition of the chromosomes at diakinesis is strikingly uniform. As a rule, all the chromosomes except one or two are in the form of rings (Fig. 5) as illustrated already by Gregory
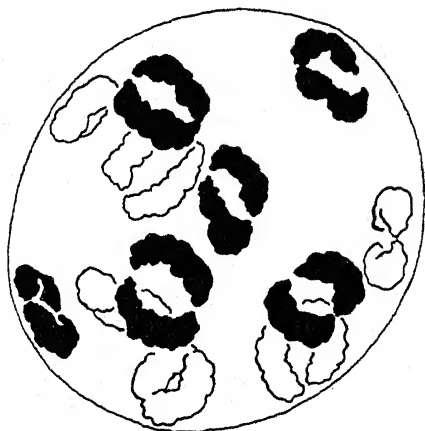
Fig. 5. Diakinesis in the diploid: twelve rings. (×5700.)

(1909). The exceptions are chromosomes associated at one end instead of at both. At early diakinesis a few subterminal chiasmata may still be seen (Fig. 4 (8) and (9)).

At metaphase the chromosomes are still further contracted, and the forms of the bivalent might easily escape observation. Side and polar
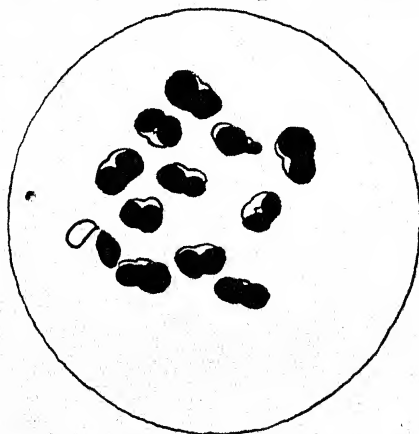
Fig. 6. Metaphase of the first division in the diploid, polar view: eleven rings and one rod.
(×5700.)

views (Fig. 6) however agree in giving from nine to twelve bivalents as
rings (with chiasmata at both ends) and from none to three rod-shaped
bivalents (associated at one end). In side views the rod-shaped bivalents
stand out from the rings most clearly by their length (Fig. 7). In polar
views the rings can be distinguished from one another by the relative sizes
of the limbs on either side of the attachment constriction (Figs. 8 and 9).

Fig. 7. Side view of metaphase of the first division in the diploid, bivalents drawn
separately: eleven rings and one rod. (× 5700.)
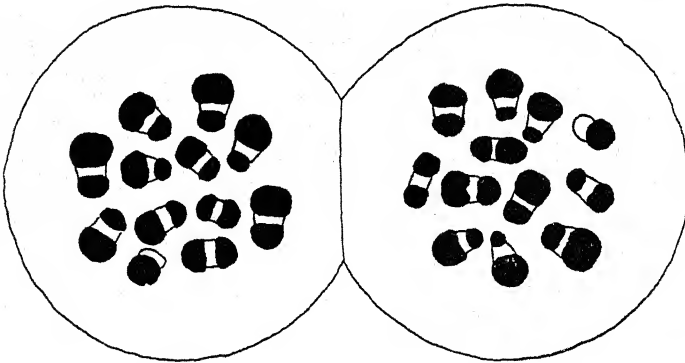
Fig. 8.   Fig. 9.

Figs. 8 and 9. Plan of metaphases of the first division in two cells of the diploid, showing
the axial part of the rings black. The relative size of the two limbs shows the position
of the attachment and the same types can consequently be distinguished in the two
divisions. (× 5700.)

At anaphase the nature of the chiasma (concealed for the moment
by the close association of metaphase) again becomes evident. The two
chromatids are often slightly separated at the tip as the chromosome
passes to the pole. The ends of the chromatids must have remained, as
they were at prophase, associated separately with their partners of the
opposite chromosome. The chromatids, therefore, *change partners* (cf.
diakinesis, Fig. 4) and the association may be properly described as a
terminal chiasma (cf. Darlington, 1929 *b*).

Table III gives a summary of a few observations of whole nuclei at
diakinesis and metaphase.

TABLE III.

Diakinesis* (22 nuclei)	No. of rings			10	11	12	Total
	No. of nuclei:	82/30		1	8	5	14
		89/30		1	5	2	8
				2	13	7	22
Metaphase (29 nuclei)	No. of rings		9	10	11	12	Total
	No. of nuclei: 280/30		2	8	17	2	29

* At the stage recorded about 1 in 30 of the chiasmata were still subterminal.

Using Fisher's tables for determining the chance of the diakinesis and metaphase observations having been taken at random from comparable material: $X^2 = 3.98$ ($n = 3$) and P = ca. 0.28. The difference is therefore not significant. The mean number of rings is 10.7 and of metaphase chiasmata 1.89 per chromosome.

IV. MEIOSIS IN THE TETRAPLOID.

The chromosomes associate in pairs at prophase apparently with exchanges of partner amongst the four homologues (Fig. 10). The quality of the fixation however does not allow of a detailed study of these exchanges, which was only possible with smears in polyploid *Hyacinthus* (Darlington, 1929 b), but the pachytene in the *Primula* always has the expected appearance of paired chromosomes like the diploid; association must therefore obey the rules made out in the earlier case. The exchanges among the chromosomes make it possible for more than two chromosomes to associate at metaphase, just as, we may say, the chiasma exchanges at diplotene make it possible for more than two chromatids to associate.

Fig. 10. Zygotene in the tetraploid, showing an exchange of partners amongst the pairing chromosomes. Without such exchanges only bivalents can be formed. (× 5700.)

At diplotene (Fig. 11) the number of loops formed is comparable with that in the diploid. That is to say, the four chromosomes form from four to ten chiasmata, or perhaps more. These chiasmata are formed between the chromosomes at random, as the exchanges of partner at the pairing stage would lead one to expect. This stage is therefore analogous in regard to chromatid relationship to the corresponding stage in triploid *Tulipa* (Newton and Darlington, 1929), and to metaphase in polyploid *Hyacinthus*[1]. As in *Hyacinthus*, certain configurations occur (Figs. 11

[1] Diplotene has not been examined in *Hyacinthus*, but there is probably no change in the relation of the chromatids between diplotene and metaphase since the chiasmata are evenly distributed along the chromosomes.

and 12) of which the interpretation is of critical importance for the theory of crossing-over. These have been discussed elsewhere (Darlington, 1930 b).

Terminalisation proceeds as in the diploid, but owing to the occurrence of chiasmata between one chromosome and several others, multiple chiasmata between several chromosomes are formed when they reach the end (cf. Diagram, Fig. 69, Darlington, 1929 c and Section V).

At early diakinesis in the tetraploid, as in the diploid, the pairing chromosomes are drawn more widely apart than at any other stage. If terminalisation is complete it is often difficult to see any connection between the chromosomes. If it is incomplete the parts of the chromosomes distal to the chiasma are joined to the rest of the chromosomes by an almost invisible thread (Fig. 14). Thus there might appear, with less favourable material, to be less association at this stage than later, with the result, as I expressed it in regard to *Prunus* (1928), that "secondary pairing" would appear not to be a continuation of a prophase relationship. In *Primula sinensis* these observations leave no doubt that "secondary pairing" at metaphase is always due to the persistence of multivalent association at prophase. The two cases are probably distinct.

At a later diakinesis stage the chromosomes are no longer widely separated and their association is, and continues to be, terminal. I have observed only one exception to this rule (metaphase, Fig. 19, p. 80), but this exception is important, for it bridges the gap between meiosis in such a form as *Primula sinensis* and the other type where interstitial chiasmata are retained at metaphase. I made the same observation in *Tradescantia virginiana* where, allowing for the great disparity in size, chromosome structure seems to be strictly comparable. We must suppose that terminalisation is suspended at a certain stage, probably owing to the degree of contraction of the chromosomes, and that contraction and terminalisation are not so perfectly adjusted as in *Oenothera*, *Rhoeo* and *Campanula* (Gairdner and Darlington, 1930). Uniform terminalisation should give the maximum regularity in the separation of chromosomes, and this must have been essential to the development of the delicate ring mechanism in these genera.

With terminal association the number of chromosome configurations possible in a tetraploid (as in a diploid) is limited. On the assumption that the pairing properties of a chromosome are specific to its parts, the number of distinguishable types of "configuration" at diakinesis or metaphase in a diploid is three, viz.: (i) chromosomes free at both ends

(unpaired); (ii) chromosomes attached at one end; (iii) chromosomes attached at both ends. There are twenty types of configuration possible in a tetraploid, of which ten are quadrivalents (*v.* Diagram I). Of the quadrivalent types, nine occur in the tetraploid *Primula sinensis*;

Fig. 11.
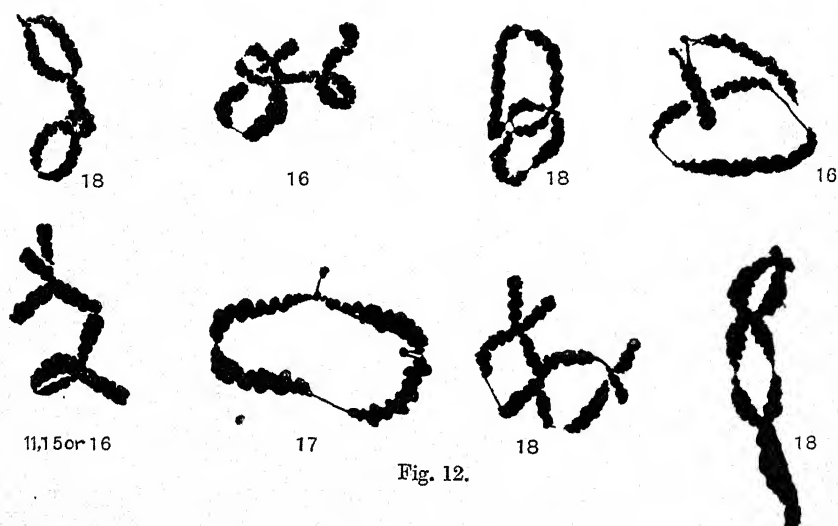
Fig. 12.

eight (including the one not found in *Primula*) have been described by Belling (1927) in tetraploid *Datura*; here also association is always terminal.

The following are the three most obvious differences in chromosome pairing between *Primula sinensis* and *Datura* tetraploids.
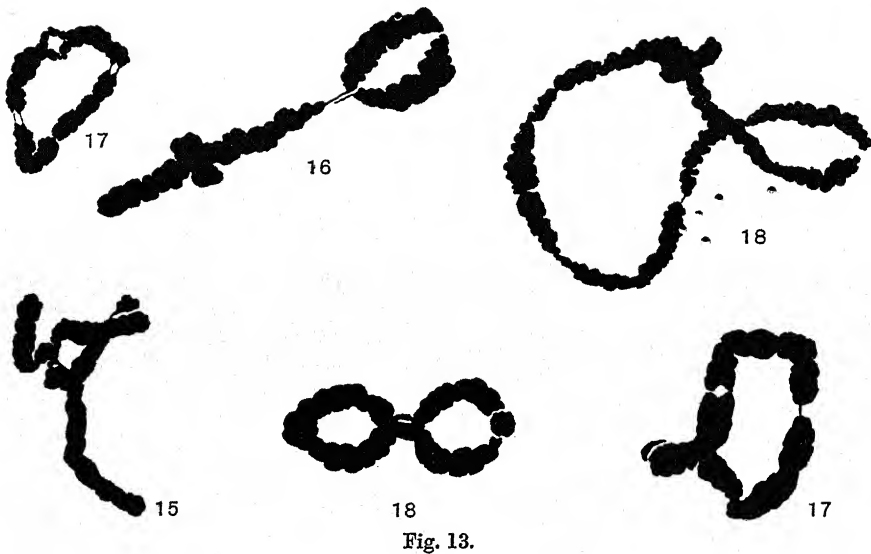
Fig. 13.
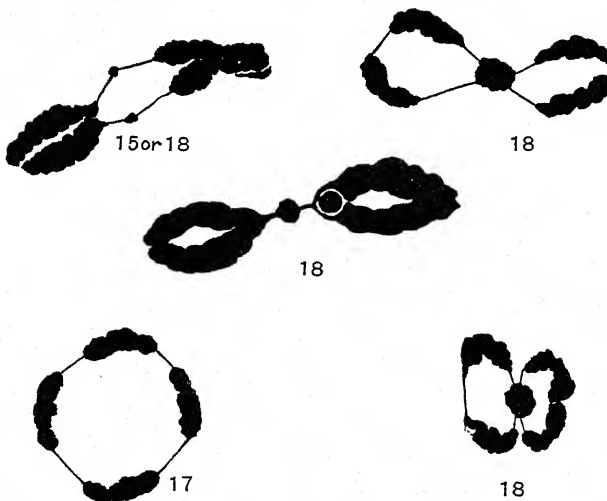
Fig. 14.

Figs. 11–14. Successive stages of terminalisation of chiasmata between diplotene and diakinesis in the tetraploid. The numbers indicate the types of metaphase configuration to which these figures would give rise (see Diagram I). The repulsion of the chromosomes for one another at the last stage apparently causes the part of each chromosome between the chiasma and the attachment constriction to be drawn into a thread (possibly a characteristic artefact). (×5700.)

<table>
<tr><td>*Primula*</td><td>*Datura*</td></tr>
</table>

(a) 1–3 quadrivalents fail	All quadrivalents formed
(b) Missing quadrivalent type requires 6 chiasmata	Missing quadrivalent types require 3 and 4 chiasmata
(c) Unpaired chromosomes occur (perhaps in 1 per cent. of cells)	Unpaired chromosomes have not been observed

CONFIGURATIONS OF CHROMOSOMES POSSIBLE IN TETRAPLOID WITH ONLY TERMINAL CHIASMATA.
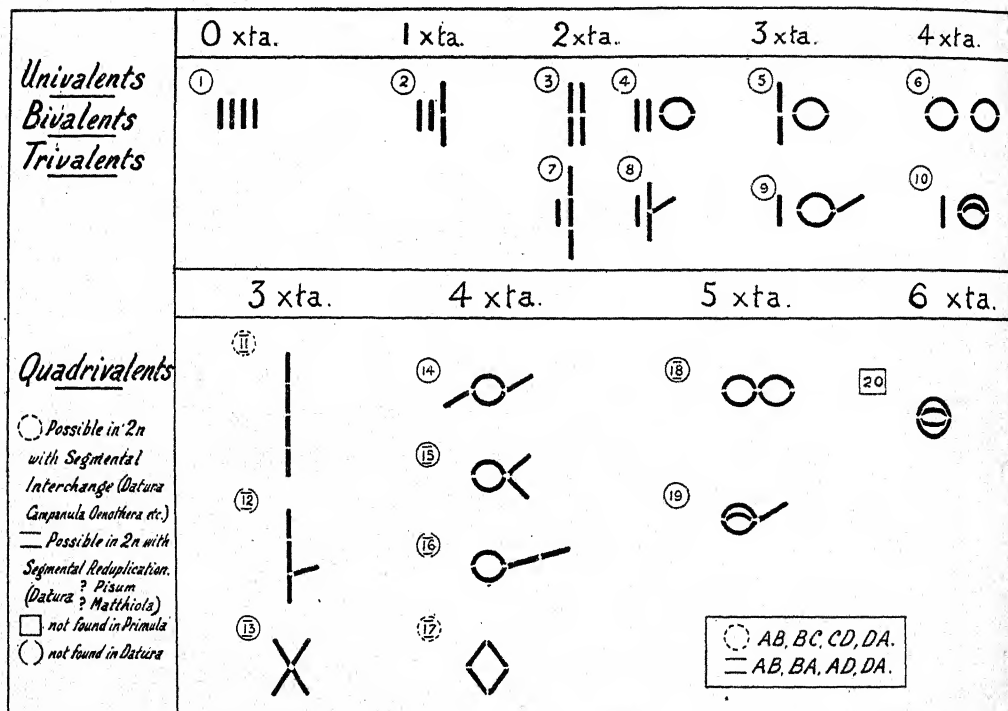
Diagram I. The twenty chromosome configurations possible in a tetraploid with terminal chiasmata are composed of six types with bivalents and univalents, four with trivalents and univalents, and ten with quadrivalents. The occurrence of these types is determined by (a) the exchange of partners amongst pairing chromosomes at zygotene, and (b) the exchange of partners amongst pairing chromatids, *i.e.* chiasmata, at diplotene. In the process of terminalisation of the chiasmata the interstitial exchanges are replaced by the simple configuration illustrated. Each of these requires a certain minimum number of chiasmata according to which the types are classified. The ten quadrivalent configurations include some that are possible in a diploid, seven with segmental reduplication and two with segmental interchange (inset formulae).

In terms of the chiasma theory of chromosome pairing, these three differences all point in the same direction: chiasma frequency is lower

in *Primula sinensis* than in *Datura Stramonium*. The fact that certain configurations are absent in the tetraploid is comparable with the fact that certain possible configurations such as unpaired chromosomes are also absent in diploids. Such configurations are incompatible with the range of frequency of chiasma formation.

It may be thought surprising that unpaired chromosomes occur in a homozygous organism, but this peculiarity is perhaps intelligible on the basis of the chiasma theory, and of my observations on *Hyacinthus*. Where the four chromosomes pair and change partners at intervals the conditions with regard to chiasma formation must be slightly different from those where only two chromosomes are involved, for the formation of a chiasma near an interchange of partners may be supposed to interfere with the formation of chiasmata in both the pairs in which the two chromosomes are associated on the other side of the exchange of partner. Such an effect would give a greater range in the number of chiasmata formed and account for the occasional occurrence of unpaired chromosomes in the tetraploid. To express this in another way: pairing in the tetraploid depends on two variables: frequency of exchanges of partner amongst chromosomes at pachytene and frequency of exchanges of partner amongst chromatids at diplotene. A wider range of variation may therefore be expected in the result.

The configurations actually seen at diakinesis are illustrated (Fig. 15). The most frequent are the ring of four (type 17), the chain (type 11) and (probably less frequent) the double ring (type 18). These require three and four chiasmata at diplotene. The other types with triple and quadruple chiasmata[1] are less frequent, and type 20, requiring six chiasmata, is absent. We may take it therefore that the average number of chiasmata persistent at full diakinesis and metaphase amongst the four chromosomes of each type is rather less than four.

At metaphase ten and eleven are the most usual numbers of quadrivalents to be found (Fig. 12); the rest of the chromosomes were bivalent in the nuclei recorded completely. Table IV shows that the mean number of quadrivalents in 21 nuclei was 10·4. Occasional univalents occur (Fig. 17). Trivalents have not been seen, but their determination would be difficult and I cannot exclude the possibility of their occurring.

Most of the quadrivalent configurations can be arranged in more than one way in regard to the type of segregation, whether, that is, even or

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[1] Resulting from terminalisation of chiasmata at different points between three or four chromosomes (cf. Diagram, Fig. 84, Darlington, 1929 c and Diagram I1, Section V).

uneven[1], disjunctional or non-disjunctional (Figs. 18 and 19).   Although, in the diploid, segregation is always even and disjunctional, in the tetraploid there appears to be little or no preference in favour of this result.
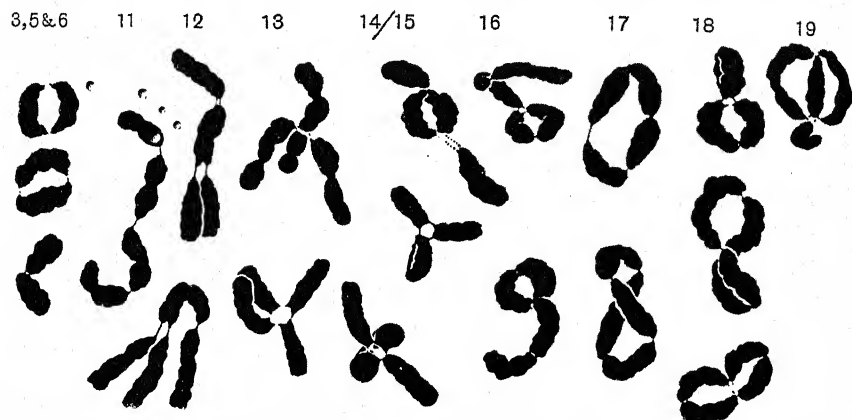
Fig. 15. Types of configuration observed at diakinesis in the tetraploid (see Diagram I).
(× 3500.)
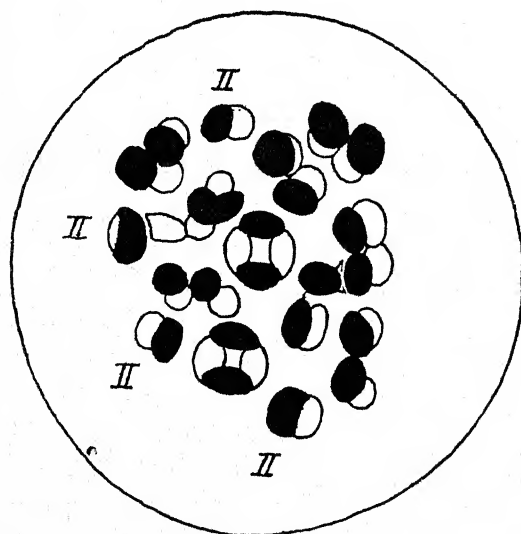
Fig. 16.

In regard to evenness, division into three and one is common in those configurations with several free ends (types 11, 12, 13, 14). In regard

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[1] Uneven numerical distribution is referred to by Belling and Blakeslee (1924) as "non-disjunction." It requires non-disjunction in a quadrivalent, but non-disjunction need not involve uneven distribution: the genetical consequences are distinct (see Section VI).

to non-disjunction, separation of paired chromosomes to the same pole is inevitable in all types with triple or quadruple chiasmata (types 12, 13, 14, 15, 16, 18, 19 and 20) and occurs also in types with simple chiasmata (types 11 and 17; Fig 18); this might be favoured by a subterminal attachment constriction. But the ring usually segregates with
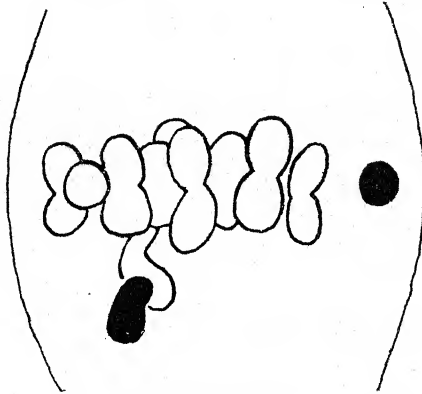
Fig. 17.
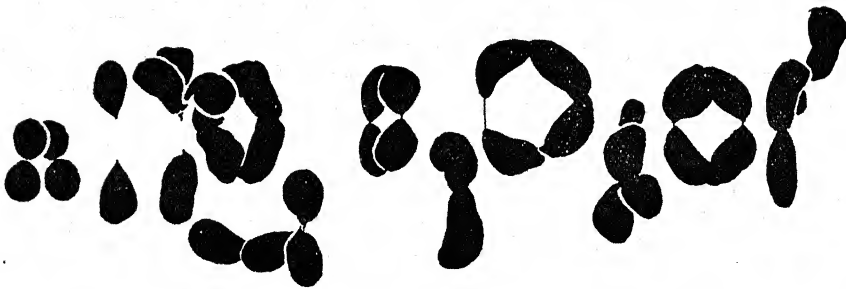
11   3   13 17   12   17   3   17   13   17   12

Fig. 18.

Figs. 16, 17 and 18. Metaphase of the first division in the tetraploid. Fig. 16, polar view, showing ten quadrivalents and four bivalents. Fig. 17, side view, showing two univalents. Fig. 18, nine quadrivalents and two bivalents in side view of one division (incomplete). Types numbered. (×5700.)

regular disjunction of pairing chromosomes to opposite poles just as in the diploid rings of *Oenothera*, *Rhoeo* and *Campanula*. In these cases the physical structure of the ring is the same as in *Primula* though its genetical structure is different. Chiasmata are terminal, spindle attachments median. Evidently, therefore, the mechanical conditions at metaphase determine the type of segregation. The genetical conditions are irrelevant.

Several of the configurations (particularly types 11 and 12) are liable to divide leaving one of their components lagging on the plate at anaphase. These, as well as univalents proper, make up the one, two or three bodies occasionally found dividing on the equator at late anaphase

11   11   11   12   13   13   13a   13a

15   16   17a   17   17   18
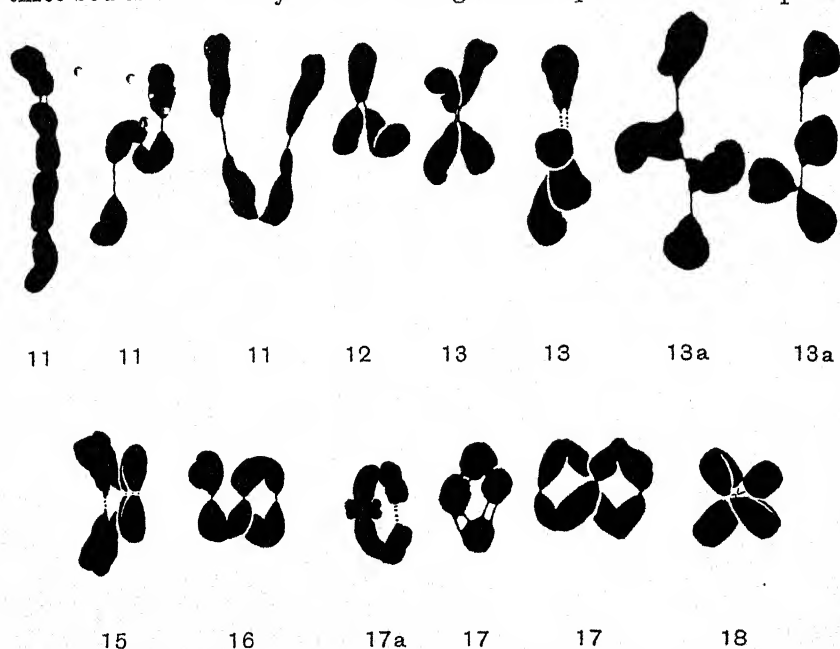
Fig. 19. Types of quadrivalent in side view of metaphase. All the types found at diakinesis except 14 and 19 have been picked out at metaphase. In 13 *a*, one connection in the quadruple chiasma has been broken; in 17 *a* one of the chiasmata is still subterminal. (×5700.)

Fig. 20. Three diakinesis-like types of daughter quadrivalent found at interphase as a result of non-disjunction. (×5700.)

after the complete separation of the bivalents. I have found similar conditions in tetraploid *Prunus* where quadrivalents are formed (1928). Following non-disjunction the associated chromosomes are seen at interphase in a condition rather similar to that at diploid diakinesis (Fig. 20).

TABLE IV.

Frequency of quadrivalent and bivalent formation in tetraploid Primula sinensis.

No. of quadrivalents	9	10	11	12
No. of bivalents	6	4	2	0
No. of metaphases	1	11	9	0

V. END-TO-END PAIRING OF CHROMOSOMES.

The type of behaviour at meiosis in *Primula sinensis* is probably very widespread in animals as well as in plants (*v.* Diagram II). It consists, according to my interpretation, in the formation of several chiasmata at diplotene which, moving away from the attachment constriction, give at metaphase simply terminal association. The attachment constriction being usually submedian, the chromosomes are attached at both ends. In the corresponding type with terminal attachment such a movement naturally means attachment at only one end.

To this class (as distinguished from that with stationary chiasmata, *e.g. Hyacinthus, Tulipa, Lilium, Chorthippus, Stenobothrus*; cf. Bĕlař, 1928) an important group of plants can probably be assigned: *Datura, Campanula persicifolia* (cf. Gairdner and Darlington, 1930), *Triticum, Oenothera, Tradescantia, Rhoeo,* and *Matthiola* (Lesley and Frost, 1927; Philp and Huskins, unpublished). Although in all these cases the chromosomes are only associated at their ends (with occasional instructive exceptions) at metaphase, we must assume that this end-to-end association is due to terminalisation of chiasmata formed at random following an earlier parallel association of homologous parts for these reasons:

(i) Wherever the chromosomes are large enough for detailed observation or their structure is revealed by multiple associations (in polyploids) the union can be seen to be of the nature of a chiasma (*i.e* a change of association amongst chromatids) and the bivalent therefore a true "tetrad."

(ii) The behaviour in *Primula* shows the origin of the multiple chiasma, as was predicted from observations of metaphase in *Tradescantia* (Darlington, 1929 *c*, Fig. 84). The occurrence of cancellation there suggested is however improbable.

(iii) In every case where the structure of the chromatids can be traced back it is found to be derived from parallel association by chiasma formation (cf. footnote 2, p. 4, Darlington, 1929 *b*).

(iv) Wherever chiasma formation has been observed it has been found to be more or less at random along the chromosome, or else in the
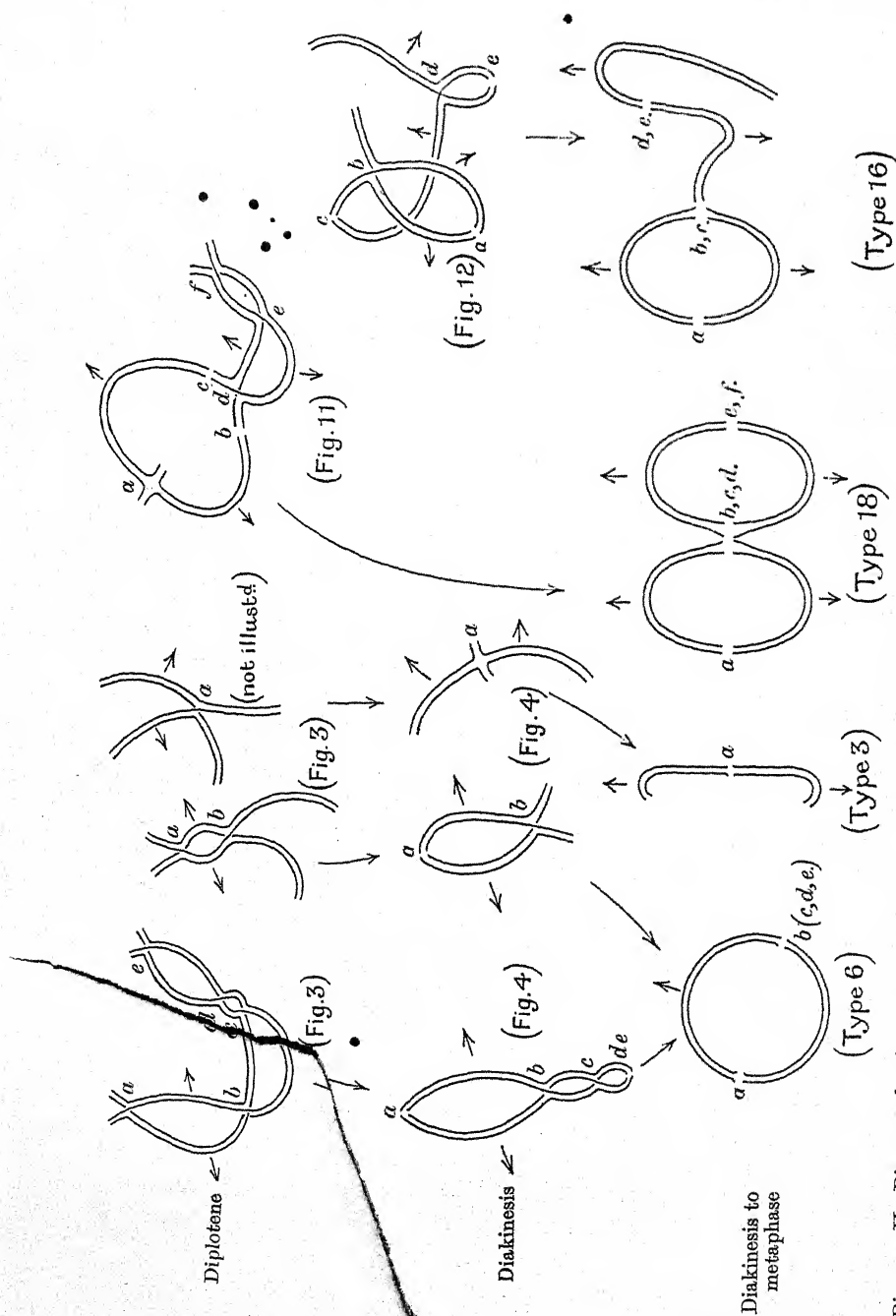
Diagram II. Diagram showing terminalisation of one to five chiasmata, as illustrated in the figures referred to, in diploid and tetraploid. The letters mark chiasmata. At the later stages both simple and multiple chiasmata may correspond to two or more earlier ones as shown. The small arrows mark the attachment constriction (not visible at the earlier stages, but known to be submedian in most chromosomes). The long arrows show the direction of the change of structure in terminalisation. The type numbers refer to Diagram I and Fig. 15. The lengths of the chromosomes are not drawn to scale but in plan.

neighbourhood of the attachment (Newton and Darlington, 1930), never entirely restricted to the two ends.

(v) The association of the ends is always found to depend on the specific and constant property of pairing of particular ends. This has been exhaustively proved by Cleland's work on certain diploid forms of *Oenothera* where exceptional conditions prevail (cf. Darlington, 1929 *a*), but the correspondence of all configurations to expectation on this assumption in other structural hybrids (*Datura* and *Campanula*, cf. Table I), in tetraploids (*Datura, Primula sinensis*), and the absence of any association in true haploids (*Datura* and *Oenothera*) is also a strong corroboration of this view.

The formation of quadrivalents in the tetraploid (see Diagram I) evidently depends on two types of observed exchange: (i) the exchange of partners between four pairing chromosomes at zygotene (Fig. 10), and (ii) the exchange of partners between four pairing chromatids at diplotene (chiasmata). The observations from Figs. 3, 11 and 12 give 3·5 as the average number of chiasmata formed per bivalent, but this is not sufficient to enable one to predict the proportion of quadrivalents without a knowledge of the frequency of the first type of exchange. Evidently however the first type of exchange takes place as a rule at least once or twice in each set of four. Where an exchange takes place, and at least three of the four combinations establish chiasmata at diplotene, a quadrivalent will be formed.

VI. SEGREGATION IN TETRAPLOIDS.

Two of the above observations affect the interpretation of genetical results. The occurrence of uneven distribution of chromosomes will result in the formation of aneuploid gametes, which evidently function on both sides (as in *Datura*, Belling and Blakeslee, 1924; cf. Fig. 2). Since this will affect a particular one of the linkage groups only once in twelve cases and then modify the phenotype in only a small proportion of these, the effect on genetic ratios will be very slight. But non-disjunction (apart from uneven distribution) will have an important effect on segregation.

The unitary organs of Mendelian segregation are the chromatids, which are in fours in the germ mother cells of a diploid, and in eights in those of a tetraploid. The two chromatids of each chromosome are identical. It follows that segregation of differences amongst the pairs in a diploid must be equational at one division and reductional at the other. The point of attachment seems to divide reductionally at the

first division in *Drosophila* (Anderson, 1925; Bridges and Anderson, 1925), *i.e.* association is between identical threads and, as this is the only part of the chromosome the association of which can be definitely determined, it is not unreasonable to suppose for the sake of argument that all association is of this kind and that differences in regard to the order of divisions are the result of crossing-over. On this view the proportion of equational first divisions of a genetic factor or a dissimilarity in the structure of pairing chromosomes (Wenrich, 1916, *et al.*) would be a measure of the distance of the locus in question from the attachment constriction. This assumption is a modification of Janssens' "partial chiasmatypy" (1924) to which I shall refer later.

In diploids the order of division is of little genetic importance except where attachment of chromosomes leads to regular non-disjunction, where several factors are determined in the haploid phase or where more than two spores of a tetrad can be isolated. But in polyploids this question is more serious. Constant association of identical chromatids at the attachment constriction will give a reductional first division and an equational second division at this point. In so far as crossing-over occurs between a given locus and the attachment, the occurrence of non-disjunction or, more correctly, the conjunction of chromosomes which have crossed over will give the possibility of a reductional second division. This possibility was invoked by Blakeslee, Belling and Farnham (1923) to account for the occurrence of recessives in the progeny of triplex plants. What they regarded as an accident it seems necessary (with the information now available) to regard as a regular phenomenon with a determinable frequency.

The occurrence of *double reduction* (Darlington, 1929 *c*, p. 244; Haldane, 1930) will naturally give a higher proportion of "homozygous" gametes from each parent, the theoretical limit being the assumption of a random segregation of chromatids considered by Haldane. There are three circumstances which will operate to restrict this randomness, viz. (i) the regular association of identical chromatids at the attachment constriction; (ii) the fact that at any one point (in *Primula sinensis* as in *Hyacinthus*, Darlington, 1929 *b*) there is no free association between eight chromatids but only between four; (iii) in certain parts of certain chromosomes non-disjunction from a chromosome with which crossing-over may have taken place probably occurs with higher frequency than in other loci.

An intermediate condition between random segregation of chromatids and diploid segregation to be expected on these grounds has been found in *Datura* in many families (cf. Haldane) and *Dahlia* (Law-

rence, 1929). If Anderson's observations on *Drosophila* are applicable, as they seem to be, it should be possible to determine the position of loci in relation to the attachment constriction and therefore to one another independently of linkage, by the freedom of chromatid assortment.

VII. THE THEORY OF CROSSING-OVER.

It is now perhaps fitting to re-examine the relationship of chiasmata to crossing-over in the light of recent observations. One hypothesis was considered in detail by Seiler four years ago, but in several respects we can now regard the question differently. It has, moreover, acquired a new importance in two ways: first, on account of the suggested relationship of chiasmata to chromosome pairing in general; secondly, on account of the relationship of crossing-over to translocation and inversion. Crossing-over is a means by which translocation or inversion can result in loss, reduplication or segmental interchange. It is therefore a more direct or at least more intelligible means of variation in relation to these changes than in relation to the less defined class of differences that we still know as "factors" or "genes."

There are two possibilities of relationship between chiasmata and crossing-over in *Primula sinensis*. Either the number of chiasmata formed at diplotene, or the reduction in numbers between diplotene and diakinesis, may be correlated with crossing-over. The second of these hypotheses is untenable, for in *Lathyrus* (Maeda, 1928, microphotograph) and in *Pisum* (Håkansson, 1928; Richardson, 1929) interstitial chiasmata are still found at metaphase. The chiasmata are therefore relatively stationary. The first hypothesis, in view of the observations (i) that crossing-over takes place between chromatids (genetically) (Bridges and Anderson, 1925); (ii) that identical chromatids are associated at the spindle attachment (Anderson, 1925, *et al.*); (iii) that the chromatids associate independently in polyploids (cytologically) (Newton and Darlington, 1929); and (iv) that association is always by chiasmata and never by interlocking (Darlington, 1929 *b*) must be a modification of the chiasmatype hypothesis of Janssens (1924) for only "partial chiasmatypy" is now conceivable.

The theoretical position has therefore been simplified and defined, and I shall consider a simple and definite hypothesis which may be stated in two ways, viz. (i) *that a chiasma is constituted by (genetical) crossing-over between two of the four chromatids taking part in it*, or (ii) *that association at diplotene is between chromatids derived from the same somatic chromosome*.

The second statement involves a bigger genetical assumption and a smaller cytological one. But since it is justified by the genetical observations as far as they go (Anderson, 1925) I shall adopt it.

The objections raised by Seiler (1926) to Janssens' chiasmatype hypothesis may be summarised under the following seven heads:

(1) Actual breaking and rejoining of threads has never been observed.

(2) The theory does not explain why exchange takes place, *i.e.* why chiasmata occur.

(3) It does not explain why the chiasmata interfere with one another, since they are determined by conditions before diplotene.

(4) It seems to require more frequent deficiency and duplication than have been found.

(5) It does not explain why only identical blocks exchange.

(6) Chiasmata are formed more frequently in a chromosome, according to Janssens, than crossing-over has been shown to occur.

(7) Conditions of chiasma formation have always been found to be similar in male and female, but in *Drosophila* crossing-over is entirely different.

The first three of these objections do not affect a simple hypothesis of correlation such as I am now considering.

The fourth and fifth objections are no longer valid. Errors in exchange would not be expected, more particularly perhaps in view of the observation that the attraction between chromosomes is specific for their parts (in triploid and tetraploid tulips and hyacinths) and not generalised.

The sixth and seventh objections require consideration.

The most definite observations of diplotene chiasmata are recorded in Figs. 3 and 11. These show an average of 3·5 chiasmata per bivalent. This may be an under-estimate, for the longest chromosomes would be most frequently cut. With a range of length from 1 to 2μ a proportionate number of chiasmata would mean a range in the average of approximately 2·33 to 4·67.

The maximum length in cross-over units to be expected would therefore be 116·7 (assuming that an average of one chiasma per chromatid concerned means 50 units).

On the genetical side, Miss de Winton tells me that a fifth factor V has been determined in the $SBGL$ chromosome with about 41·3 per cent. of crossing-over from L. There is also in the same chromosome a sixth factor X between B and G which has not yet been mapped exactly. Let

us take with this the crossing-over figures given by Miss de Winton (1927):

$$V\text{--}S = 41\cdot3 \text{ per cent.}$$
$$S\text{--}B = 12\cdot5 \quad \text{,,}$$
$$B\text{--}G = 34\cdot5 \quad \text{,,}$$
$$G\text{--}L = 1\cdot8 \quad \text{,,}$$

for these Haldane's (1919) corrected distances in centimorgans are $55\cdot0 + 13\cdot0 + 41\cdot8 + 1\cdot8$: total $111\cdot6$. This chromosome may be provisionally and approximately represented thus:

ERRATA

p. 86, l. 35:
    for 116·7 *read* 116·7 to 233·4.

p. 86, ll. 35–36:
    for chromatid *read* chromosome.

p. 87, l. 12:
    for 58·3 to 116·7 *read* 116·7 to 233·4.

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number of cross-overs is unrelated to the study of their mean number.

The seventh objection was, and remains a serious one. It will now repay consideration.

VIII. Pairing and crossing-over in *Drosophila*.

Cytological observations of *Drosophila* have not so far yielded definite results on the structure of the paired chromosomes, and the recent observations of Metz (1927) and Guyénot and Naville (1929) seem less decisive in this respect than the earlier studies of Stevens (1908). On the chiasma theory of chromosome pairing we can study chiasma formation

from another and unrelated point of view: we can examine (i) the regularity of disjunction of chromosomes in the diploid, and (ii) the method of segregation of chromosomes in the polyploid. I shall, therefore, approach the question in the first instance from the genetical side.

In the first place Stern's recent summary of observations on reduction of the XY chromosomes in *Drosophila* (1929 b) is of critical importance considered on the basis of the chiasma theory of chromosome pairing. He shows, *inter alia*, that (i) Y'' behaves just like Y in regard to the pairing with X (this I take to mean that the distal arm of Y is not concerned with pairing with X); (ii) Y competes unequally with each X in pairing with the other X (this I take to mean that Y pairs with X along a shorter distance than that along which two X's pair)[1]; (iii) X competes equally with each Y in pairing with the other Y (this I take to mean that X pairs with Y along the same distance as that along which the Y's pair, at least in the male); (iv) Y segregates at random in the presence of Y' (this I take to mean—although this case is a complex one— that Y' has lost the part of chromosome in which Y normally pairs with X and the free Y pairs equally with the free and attached X's). These conclusions are worth considering in the absence of direct evidence. It seems possible that the pairing of X and Y is determined by two conditions; first by the fact that X and Y only correspond for a short distance, and secondly by the possibility that chiasma formation is always confined to one region in the male. Obviously the first of these conditions might be derived from the second for, in the absence of crossing-over, the two sex chromosomes will vary independently[2]. This may be taken to account for the observations, generalised by Haldane (1922) and Huxley (1928), that crossing-over is so often less in the heterogametic than in the homogametic sex.

We must therefore assume that (i) the chiasmata are restricted to one region in the male, but not in the female, and that (ii) they are reciprocal,

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[1] This conclusion is also suggested by Metz's observation (1927). In both cause and effect this differential affinity is analogous to that shown in diploid and tetraploid *Primula kewensis* (Newton and Pellew, 1929). The failure of the XXY trivalent, although the XX bivalent never fails, is partly analogous to the failure of the short trivalent in *Hyacinthus* (Darlington, 1929 b).

[2] Where two homologous chromosomes both essential to the preservation of the species are prevented from crossing-over along a part of their length they will vary at random along that part, in which they will thus come to differ sufficiently to prevent crossing-over mechanically were it permitted genetically. This I take to be the condition in the X–Y pair in *Drosophila* and (although in this case structural change has perhaps anticipated restriction of crossing-over) in pairing chromosomes of opposite complexes in the ring-forming races of *Oenothera*.

where interstitial, in the male[1]. These assumptions correspond to the observations of crossing-over if we take the crossing-over to be confined in the male to a region in which no factors are situated. The first assumption corresponds to the observation of a cytological difference between two groups of species in *Fritillaria*, one like *F. Meleagris* (Newton and Darlington, 1930), in which chiasmata are formed only in the neighbourhood of the attachment constriction, and the other like *F. imperialis*, in which, I find, the chiasmata are formed all along the chromosomes though with different frequencies in different clones.

It also probably corresponds to such a difference as that observed by Janssens (1924) between *Mecostethus* and *Stenobothrus*, the one having localised, the other distributed, chiasmata. It is in *Fritillaria* however that the conclusion is so obvious that the difference is a genetical one to which the chromosomes are indifferently subject. This is a fundamental point of agreement with the observations of crossing-over, in considering which Haldane has remarked (1922) that "the same mechanism which prevents them [the sex factors] from crossing-over may be expected to hinder or prevent crossing-over of all factors in that sex." (Cf. Detlefsen and Clemente, 1923.)

It may seem far-fetched to compare the differences between the two sexes of one species with the differences between two species, all of whose corresponding chromosomes differ. But while the autosomes of the two sexes are not divergent, about one-quarter of the hereditary material in *Drosophila* has been undergoing unrelated differentiation in the two sexes for a period greater than that allowing the differentiation of species or even genera. Hence the resultant difference of balance between the sexes should be at least of the same order as that between species.

Turning to the cytological observations we find little definite evidence of the kind necessary to prove or disprove the hypothesis of chiasma localisation. It must be borne in mind, however, that the determination of this peculiarity in *Fritillaria* was only arrived at after some years of study and could only be placed beyond dispute by the comparison of chromatid structure at metaphase with that at the stages before and after. This is in spite of the fact that *Fritillaria* provides some of the best material for the study of meiosis to be found in plants. Its chromosomes are two or three hundred times bigger than those of *Drosophila*.

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[1] Two other hypotheses that I have considered with a view to applying them to the differences between the sexes are (i) that crossing-over is due to breakage of chiasmata in terminalisation, and (ii) that chiasmata might occur between identical chromatids, *i.e.* equationally (Darlington, 1929 *b*). Both these hypotheses may now be dismissed.

In *Prunus* I have recently been able to make out the chromatid structure in chromosomes of the same size as those in *Drosophila* and having interstitial chiasmata at metaphase (1930 *a*). But *Prunus* in spite of the size of the chromosomes is favourable material. Nevertheless it seems possible that Stevens' figures, particularly 65, 70 and 72, may be given a compatible interpretation. They do not definitely decide whether the attachment constriction is between or outside the reciprocal chiasmata, although the autosomes in Fig. 71 indicate that it is outside.

Let us consider this hypothesis in greater detail. (i) We must assume a difference in chiasma formation such as that shown in Diagram III. (ii) The difference must naturally be determined by genetic factors inseparable from those of sex in so far as the crossing-over conditions are sex-limited, and not determined by the $X : Y$ proportion (Stern, 1929 *a*). (iii) The Y chromosome will be limited in its pairing with the X, even in the female, by the dissimilarity that has developed between them in consequence of the concentration of crossing over. (iv) Only two chiasmata may be formed in the neighbourhood of the attachment constriction in the male, or in the Y chromosome in either sex, and they must always be *reciprocal*. The last of these assumptions is new; it can, however, be tested to some extent by the detailed consideration of certain genetical data.
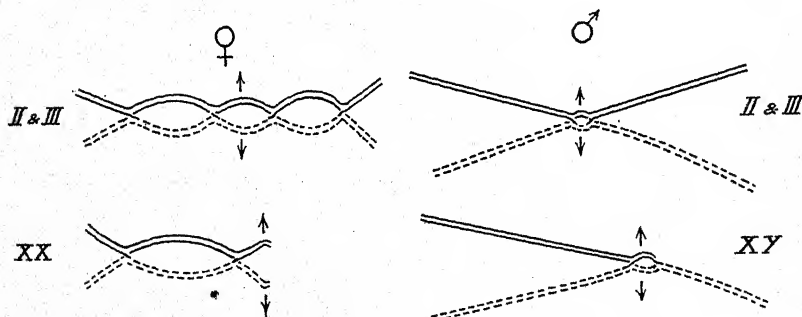
Diagram III.

(i) Bridges (1916) finds 92 per cent. of $X : XY$ distribution and 8 per cent. of $Y : XX$ distribution in an XXY female. In the former type crossing-over between the two X's is equal to that occurring in the normal diploid female. In the latter type no crossing-over occurs between them (Bridges, 1916; Morgan, *et al.* 1925). There must therefore be only XY bivalents in this type, and if XXY trivalents are formed they must always segregate $X : XY$. This is not surprising, for with a chiasma on

either side of the attachment in the XY pair the two attachments would be very close together and the two chromosomes might well pass together to the pole. Since it is impossible to say what proportion of the 84 per cent. of $X : X$ disjunction is derived from bivalents, it is also impossible to say that the segregation agrees with expectation on the hypothesis, except in a general way. But it may be considered that the fact that pairing of Y with an X prevents the pairing of two X's in 16 per cent. of cases without definitely reducing crossing-over between them is an objection to the hypothesis. If so it would be an objection to the established view that crossing-over depends on pairing. But since crossing-over conditions are so different in disomic and trisomic races for reasons that we can only partly understand the two types can scarcely be compared.

(ii) Stern finds that the $\widehat{XY'}$ attached chromosome type arises once in 1500 to 2000 times, *i.e.* much more frequently than the attached X. This is comparable with the frequency of occurrence of half-mutants in *Oenothera Lamarckiana*. Now the regular pairing of chromosomes at zygotene is naturally assisted by the homologous pairing parts being of great length, for irregularity is impossible at one point if at points on either side of it pairing is normal. The possibility of chromosomes pairing and exchanging in an inverted relationship with one another should be much greater on the present assumption between X and Y than between two X's. The occurrence of the attached Y chromosome may be attributed to such abnormal crossing-over, on our assumption, for an inverted chiasma could not be balanced by a reciprocal, and being single would necessarily produce this result. Furthermore, this conclusion is in accordance with the long arm of the Y and not the short being attached to the X if we regard the long arm containing the *bobbed* inhibitor as being primitively homologous with the X containing *bobbed* in a corresponding region.

(iii) Stern has examined the possibility of crossing-over between the doubly heteromorphic chromosomes $\widehat{XY'}$ and Y''. On the above hypothesis of $\widehat{XY'}$ being the result of inverted crossing-over, this chromosome would be disomic in the pairing region. $X\widehat{XY'}Y''$ females would be tetrasomic and the homologous regions of the attached chromosomes would naturally pair most frequently with one another. Crossing-over would not therefore take place. $\widehat{XY'}Y''$ males, on the other hand, would be trisomic. Whenever Y'' paired it would pair with one of the reduplicated regions in competition with the other. Such circumstances would occasionally compel the occurrence of a single (non-reciprocal) chiasma. Thus the absence of crossing-over in the female and its rare occurrence as the "reorganisation" found by Stern in the male might

be expected on this hypothesis. On the theory that meiosis is determined by the precocity of the prophase (Darlington, 1930 *c*) it is possible to imagine the occasional occurrence of meiotic conditions in somatic divisions. These would scarcely be likely to be regular in their effects, but might lead to chiasma formation and crossing-over of an abnormal kind. In a word, such conditions might make for the occurrence of a non-reciprocal chiasma. Such would be the explanation on the present hypothesis of exceptional crossing-over in the male as found by Muller (1916, p. 304). Unfortunately the position of the attachment has not yet been located as exactly in the second chromosome as in the first and third, so that it is not apparently possible to say whether it lies between "truncate" (13) and "black" (48·5), *i.e.* whether the crossing-over was in one of what I am supposing to be the normal positions.

(iv) A second important source of evidence on the basis of crossing-over seems to be provided by Gowen's observations on a crossing-over suppressor in the female *Drosophila* (1928, *et al.*). He observes (genetically) that this suppressor causes irregularity in meiosis in the female, although not in the male. He concludes that "these results show clearly that the chromosomes of male and female *Drosophila* pass through phases which must be divergent in at least two particulars, chromosomal linkage and disjunction." But does this necessarily follow? On my theory of chromosome pairing (1929 *b* and *c*) failure of pairing results from failure of chiasma formation. In these terms, therefore, Gowen has observed a correlation of failure of chiasmata with failure of crossing-over—a substantial contribution to the chiasmatype theory. That the irregularities should not appear in the presence of the accurately adjusted male factor for crossing-over localisation is not surprising and seems to show rather that the same result may arise in two ways, the wild type character being epistatic to the mutant.

Similar results have been obtained by Bridges (1929). With incomplete suppression of crossing-over the same loss of fertility occurred. But it was also found that crossing-over was slightly increased in the mid-regions of the third chromosome at the same time that it was reduced in the distal regions. Again Detlefsen and Clemente (1923) found an increasing reduction in crossing-over in the X chromosome, moving away from what is now known to be the spindle attachment (viz. 30·3, 60·1, 68·7, 82·3 per cent. in successive regions). It will be observed that these are the differences that occur between chiasma distribution in the two species of *Fritillaria* and the aberrant form is half way towards what I am supposing to be the condition in the male.

I call attention to these considerations merely to show the direction in which further enquiry may throw some light on the relationship between chromosome behaviour and crossing-over in *Drosophila* and elsewhere. A number of hypotheses could be suggested, to account for differences between male and female, of which this is, for the moment, the most plausible.

IX. SUMMARY.

1. Mitosis and meiosis are described in diploid and tetraploid *Primula sinensis* ($n = 12$).

2. The chromosomes are associated by terminal chiasmata (chromatid exchanges) at metaphase of meiosis in both forms. Both possible types of bivalent and nine of the possible types of quadrivalent are formed.

3. The mean number of ring bivalents is 10·7 in the diploid.

4. The mean number of quadrivalents is 10·4 in the tetraploid.

5. The metaphase configurations (mean number of chiasmata in the diploid, 1·89) result from the terminalisation of 1 to 5 interstitial chiasmata formed at diplotene (mean number, 3·5) amongst chromosomes paired simply in the diploid and paired with exchanges of partner among the four in the tetraploid. Quadrivalent formation is determined by the occurrence of these two types of exchange.

6. These observations are shown to require a modification of the classical view of segregation as applied to tetraploids. The modification agrees with observations in *Datura* and *Dahlia*.

7. They are also shown to be applicable to the study of chromosome behaviour in *Datura*, *Campanula* and *Oenothera*.

8. They agree with the genetical observations of crossing-over on the view that chiasmata are determined by a break and exchange between one pair of chromatids; that association is always, therefore, between identical chromatids. A purely cytological demonstration of this has been given elsewhere (Darlington, 1930 *b*).

9. Where crossing-over is different in the two sexes the difference in chiasma-formation between them must be analogous to differences observed between species: the chiasmata must be localised in the heterozygous sex. Where crossing-over is abolished in one sex such localised chiasmata must be reciprocal (Diagram III).

10. These assumptions are supported by observations of changes in crossing-over distribution and the occurrence of non-disjunction in association with crossing-over reduction and suppression and by obser-

vations of non-disjunction frequencies in polysomic *Drosophila*. This modification of the chiasmatype theory may therefore be considered compatible with precocity theory of meiosis (Darlington, 1930 *c*).

I am indebted to Prof. J. B. S. Haldane for valuable criticism, to Miss de Winton for the genetical results and the cytological material, and to Mr J. Philp for determination of the somatic chromosome numbers recorded from crosses.

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STERILITY AND INCOMPATIBILITY IN DIPLOID AND POLYPLOID FRUITS.

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INTRODUCTION.

EXPERIMENTS in heredity and sterility of a number of cultivated fruits have been in progress at this Institution since 1911, and reports on various aspects of the investigations have been published from time to time (1918–29). The experiments have been continued on trees grown in pots, under the methods of control described in earlier papers.

The investigations are naturally prolonged by the length of time which elapses from one generation to another, and limited by the space occupied by a family of even moderate size. We have ventured, therefore, at this stage to analyse genetically such of our results as relate to the behaviour of incompatibility.

MATERIAL.

The following are fruits used in these investigations and their chromosome numbers. The actual varieties and seedlings studied are subsequently referred to in detail.

<div align="center">Chromosome No.</div>

Prunus avium	(Sweet cherry)	16*	$2n$	Diploid
P. cerasus	(Sour cherry)	32	$2n$	Tetraploid
P. cerasus × avium	(Seedlings)	24	$2n$	Triploid
P. cerasus × avium	(Duke cherries)	32	$2n$	Tetraploid
P. domestica	(European plum)	48	$2n$	Hexaploid
Pyrus malus	(Apple)	34	$2n$	Partly tetraploid and partly hexaploid
Pyrus malus	(Apple)	51	$2n$	Partly hexaploid and partly nonaploid

* Among the cultivated sweet cherries extra chromosomes beyond the diploid number have been observed (Darlington, 1928, 1930).

INCOMPATIBILITY.

Cherries.

In Table I are given the varieties of the sweet cherry—*P. avium*—used in the experiments and the compatible and incompatible combinations so far elucidated: + = compatible; − = incompatible combinations.

With the possible exception of Black Tartarian "D," a tree first used this year, there is no question of any of the varieties mentioned in Table I not being distinct, but the identity of some remains obscure. Different individuals are frequently met with under the same name, and to find one variety under two or more names is also common. It will be noted that four individuals bear the name of Black Tartarian and are de-

TABLE I.
Males.

Table I. Males (columns, left to right): Bedford Prolific, Black Eagle, Tartarian "A", Tartarian "B", Early Rivers, Knight's E. Black, Roundell, Schrecken, Frogmore, Belle Agathe, Waterloo, Cluster, Black Heart "B", Napoleon, Emp. Francis, Kentish Big, Ludwig's Big, Late Black, Turkey Heart, Governor Wood, Elton, Hedelfingen, Mezel, Ursula Rivers, Noir de Schmidt, Peggy Rivers, Jaboulay, Tartarian "D", Belle d'orleans, Black Heart "A", Tartarian "E", Bohemian Blk "D", Early Purple, Florence, G. d'annonay, Noble, Noir de Guben, White Heart. Females (rows): the same 38 varieties in the same order. The self-incompatible and intra-sterile inter-fertile groups are shown as boxed blocks of dashes (−) along the diagonal, with plus signs (+) marking inter-fertile crosses.

signed "A," "B," "D" and "E," and two Black Heart, "A" and "B"; they are all distinct. Our Hedelfingen is not the true variety.

It will be seen that all the varieties have proved to be self-incompatible and that 29 of the 38 varieties investigated fall into nine intra-sterile inter-fertile groups. Groups 8 and 9 were only found this year and the pollinations require repetition, but from general experience we have little doubt of their validity.

The gross totals of (a) self-pollinations, (b) cross-incompatible pollinations and (c) cross-compatible pollinations made between the varieties in Table I, and the results obtained are summarised in Table II.

TABLE II.

	No. of flowers pollinated	No. of fruits set	Percentage set
Self-pollinations	33,500	35	0·1
Cross-incompatible pollinations	26,826	35	0·13
Cross-compatible pollinations	72,573	18,777	24·4

In this and all subsequent summaries "number of fruits set" means the number which reached maturity.

It is shown in Table II that a few fruits have been obtained both from selfing and from cross-incompatible pollinations. Possibly they are the result of accident but, on the other hand, occasional fruits have set under very stringent conditions, which suggests that as a rarity a pollen tube travels the full length of the style and effects fertilisation. It is hoped that when seedlings raised from these occasional fruits reach maturity the behaviour of pollinations between them and their parents may afford evidence as to their origin.

In Table III are the results obtained from pollinations between a number of seedlings, and between them and their parents.

Details of the pollinations in which *seedlings* have been used in Table III are as follows: compatible pollinations, 4808 flowers: 1091 fruits = 22·7 per cent. set; incompatible pollinations, 972 flowers: 1 fruit set.

Analysis of Tables I and III shows that so far as our investigations have progressed, the behaviour of incompatibility in the sweet cherry is comparatively simple. Self-incompatibility is the rule, cross-incompatibility common and always reciprocally expressed. Among cultivated varieties nine intra-sterile, inter-fertile groups have been recognised, and the preliminary investigations with seedlings have also given results which are orderly and amenable to the genetic interpretation of incompatibility as advanced by East and Mangelsdorf (1925, 1926) in *Nicotiana*, by Lehmann (1926) in *Veronica*, and to some extent by Sirks (1926 a, b) in *Verbascum*. By taking advantage of the end-season (pseudo) fertility which occurs in *Nicotiana*, East obtained individuals which were compatible in one way of a cross but incompatible in the reciprocal, *i.e.* by selfing S_1S_2 he obtained S_1S_2, S_1S_1, and S_2S_2 individuals. The homozygous forms used as males fail on their mother, but the reciprocal combinations are effective. Sirks, working with *Verbascum*, obtained many examples

TABLE III.

Column groups (top, with subscripts):

- $S_1 S_2$: Bedford Prolific., Black Tartarian "B", Early Rivers., Knights E. Black.
- $S_1 S_3$: Big. de Schrecken., Belle Agathe., Waterloo.
- $S_x S_3$: Big Napoleon., Emp. Francis.
- $S_3 S_4$: Big. Kentish., Turkey Heart., Elton.
- $S_3 S_4$: Gov: Wood.
- $S_3 S_4$: Big d' Schrecken × Gov: Wood. (columns 41 47 51 54 43 44 45 46 48 49 50 52 56 57)
- $S_3 S_4$: (columns 57 56 52 50 49 48 46 45 44 43 54 51 47 41)

Row labels (right side):

Bedford Prolific.
Black Tartarian "B"
Early Rivers.
Knights E Black.
Big. de Schrecken.
Belle Agathe.
Waterloo.
Big Napoleon.
Emp. Francis.
Big Kentish.
Turkey Heart.
Elton.
Gov: Wood.
41
47
51
54
43
44   Big d'
45   Schrecken.
46   ×
48
49   Gov: Wood.
50
52
56
57
125
126
122 } B. Schrecken
127 } ×
128 } Knight's Blk.
129
130
314 } B. Schrecken.
320 } × Tartarian. B"
226 } Em. Francis
227 } × Tartarian. B"
536
542
548 } Turkey Heart.
549 } ×
550 } Early Rivers
551
555

of one-way incompatibility, especially during the early stages of his investigations, and East (1929) suggests that this may be due to pseudo-fertility. We feel, however, that the frequency of the reciprocal differences and other complications encountered by Sirks are significant, and may possibly be the result of a polysomic condition of the factors determining incompatibility in *Verbascum*. Consequently we have ventured to refer to his results in more detail when discussing the behaviour of incompatibility in the polyploid fruits.

In the sweet cherry we have begun a factorial analysis of incompatibility by determining the behaviour of pollinations among the progeny of intergroup crosses.

Reference to Table III shows that Big. de Schrecken × Governor Wood gives two (intra-sterile, inter-fertile) groups, one of which fails with its male parent, Governor Wood. Two groups will appear in F_1 when the parents have a factor in common and, following East's terminology, we have provisionally assigned the factors S_1S_3 to Big. de Schrecken and S_1S_4 to Governor Wood.

A similar result was obtained from the crosses Big. de Schrecken × Knight's Early Black and Big. de Schrecken × Black Tartarian "B" (both male parents coming from Group I), which indicates that either S_1 or S_3 is common to the three parents and their respective groups. Pending further work we have assigned the factors S_1S_2 to Group I.

Three other failures with seedlings are of interest in that they disclose the identity of a factor in Groups III and IV. Seedling 226 (Emperor Francis III[1] × Black Tartarian "B" II) fails as male on Big. de Schrecken II. Since Black Tartarian "B" is S_1S_2 it follows that Emperor Francis contributed the S_3 factor.

Seedlings 536 and 542 (Turkey Heart V × Early Rivers I) failed on Big. de Schrecken II. Early Rivers I is S_1S_2 and Big. de Schrecken II S_1S_3; therefore Turkey Heart V must have contributed an S_3 factor. This is supported by the failure of Turkey Heart on Seedling 56, and it seems probable that Group V can, in its relations to the other groups, be designated S_3S_4. It is of course evident that the factors assigned to the groups are provisional, but each new factor disclosed aids in the final elucidation of the incompatible groups. Of the 38 varieties tested, at least 13 different combinations and a minimum of 6 incompatibility factors are necessary to interpret the results so far obtained.

In the sour cherries (*P. cerasus*) and the Duke cherries complete and varying degrees of self-compatibility occur. Our results with these—the

The roman numerals refer to the incompatible groups to which the varieties belong.

tetraploid cherries—have already been published in detail (Crane and Lawrence, 1929).

<div align="center">Plums.</div>

We have investigated 52 varieties of plums (Crane and Lawrence, 1929), *P. domestica*, and from the results obtained they can be primarily classified as follows.

    (1) Self-compatible.   (20 varieties)
    (2) Self-incompatible   (23   ,,   )
    (3) Partially self-compatible (29   ,,   )

Cross-incompatibility has only occurred among the varieties within Classes (2) and (3). The pollen of self-compatible varieties is always effective on self-incompatible kinds. In the sweet cherry reciprocal pollinations have always given similar results, but in *P. domestica* incompatibility occurring in one way of a cross and compatibility in the other is common.

The partial compatibles constitute a definite class setting about 3 per cent. of fruit with their own pollen and the same proportion in the partial compatible crosses. In compatible crosses they set a full crop.

In Table IV are shown the cross-incompatible combinations we have found in plums:

+ = compatible, ∔ = partially compatible and — = incompatible.

Compared with the sweet cherry not only are the results much more complex, but fewer groups occur, and those found include self-incompatible, self-compatible, partially self- and cross-compatible, cross-compatible, cross-incompatible, and reciprocal differences involving both partial and complete incompatibility.

A summary of the results from pollinations made in plums is given in Table V.

Analysis of Table IV shows that many of the varieties and seedlings must be similar in constitution with respect to incompatibility factors. For example, the results from pollinating seedlings 1024, 1029 and 1027 with Jefferson, their male parent, indicate that these individuals differ by a factor or two at the most. The established varieties, *e.g.* (1) Blue Rock and Early Rivers, and (2) Cambridge Gage, President and Late Orange are further examples of this.

Seedling 969 is noteworthy, since it is a self-incompatible seedling from a self-compatible mother, Denniston's Superb, a result which suggests that Denniston's carries at least three factors for incompatibility.

The degrees of fertility and the complexity of results in *P. domestica*

are undoubtedly due to its hexaploid constitution and manner of chromosome pairing (Darlington 1930). In a diploid each gamete carries but one incompatibility factor, and no plant produces more than two types of

TABLE IV.

TABLE V.

	No. of flowers pollinated	No. of fruits set	Percentage set
Self-incompatibles—selfed	24,638	21	0·08
Cross-incompatibles—crossed	9,032	26	0·28
Partial compatibles—selfed	38,950	989	2·6
Partial compatibles—crossed	2,614	71	2·7
Compatibles—selfed	29,806	6,819	22·8
Compatibles—crossed	48,256	13,552	28·0

gametes. In a hexaploid, however, each gamete may carry three incompatibility factors, and the number of gametic types will be greatly

increased. Hence a greater variability in the behaviour of incompatibility is to be expected in polyploid plants, and the chances of individuals of similar constitution meeting to form incompatible groups is correspondingly lessened.

The more complex the polyploidy the less frequently will complete incompatibility occur, and an examination of our results shows that incompatibility is not so common in the hexaploid plums as in the diploid cherries, and even less frequent in the more complexly polyploid apples.

We have remarked on the frequent occurrence of one-way incompatibility in the plum. This probably occurs between plants which are of a similar constitution, except that one carries another factor. Thus in a hypothetical hexaploid cross, $S_1S_1S_1S_2S_2S_2 \times S_1S_1S_2S_2S_3S_3$, those gametes of the male which carry S_3 might be expected to function, whereas in the reciprocal cross the gametes of the male carrying no factors different from the female might be expected to fail.

Similarly in a cross between tetraploids, as for example $S_1S_1S_2S_2 \times S_1S_1S_2S_3$, it is conceivable that the plant carrying S_3 might be effective as male, whereas the reciprocal combination would fail.

It seems clear, therefore, that one-way incompatibility is a phenomenon directly attributable to the multiplicity of factors in a polyploid. It has not been observed in known diploids except under the circumstances in *Nicotiana* we have previously detailed, and we suspect that its frequent occurrence in *Verbascum phoeniceum* and other plants *e.g. Cardamine pratensis* (Correns, 1912), is an indication that they are polyploid species[1]. In the Scrophulariacea eight is a common basic chromosome number, and Håkansson (1925) has shown that *V. phoeniceum* has 32 chromosomes, which suggests that it is a tetraploid.

We have mentioned that self-compatible varieties have always proved compatible as males and females with all other classes. Theoretically, however, it is to be expected that self-incompatible individuals will be found which will fail as males on self-compatibles.

For example, it is probable that, in addition to fertility factors, Denniston's Superb carries three incompatibility factors, and an individual whose constitution included no factors other than those three

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[1] A difference in reciprocal pollinations in the sweet cherry has been reported by Gardner, *Bull.* No. 116, Oregon Agric. Exper. Sta., 1913. Gardner, however, points out that definite conclusions should not be drawn from a single season's work, and since this is the only example of one-way incompatibility reported in the sweet cherry we feel that repetition is desirable.

would fail on the self-compatible Denniston's. East and Yarnell (1929) have shown in *Nicotiana* that there is a self-fertility factor, S_f, allelomorphic to the incompatibility factors S_1S_2, etc. Possibly this is also true of the plum, since self-compatible varieties have segregated self-incompatibles in their progeny.

In this account of plums and cherries we have dealt with incompatibility only, but among the varieties referred to varying proportions of defective pollen and ovules occur. We have, however, previously shown that the degree of generational sterility in these varieties is not sufficiently high to prevent a satisfactory yield in compatible combinations.

Apples.

Cytologically, cultivated apples are of two kinds, the so-called "diploids" and "triploids" with 34 and 51 chromosomes respectively. Darlington and Moffett (1930) have shown that the "diploids" are secondary polyploids, being hexasomic in respect of three chromosomes and tetrasomic in four. Thus the varieties referred to as "triploids" are partly hexasomic and partly nonasomic.

We have investigated forty varieties of cultivated apples, and among them varying degrees of self-incompatibility occur, but only two have entirely failed upon selfing and three upon crossing. Since comparatively few flowers were used in these five pollinations, too much importance cannot be attached to them.

Among the triploid apples a high degree of generational sterility prevails, and the proportion of good pollen varies from 4 to 27 per cent.; whereas that of known diploids ranges from 50 to 97 per cent.

In apples we have made a total of 290 different self- and cross-pollinations involving 40,000 flowers. An analysis of the cross-pollinations shows that triploid-diploid combinations are as productive as diploid-diploid. The seed content of the fruits of these two series of crosses is, however, markedly different, and reveals a higher degree of generational sterility operating in triploid-diploid and reciprocal combinations. The offspring of the triploid-diploid crosses are also extremely weak, due to aneuploidy, and contrast sharply with the vigorous diploid-diploid progeny.

Since incompatibility is due to lack of genetic differentiation it is concluded that the good results obtained from the triploids are due to a greater variety in the gametic output of triploid than of diploid varieties, thereby providing a greater chance of compatible combinations. The

rarity or absence of complete incompatibility and the common occurrence of partial compatibility in apples is doubtless associated with the complexity of their chromosome constitution.

Fuller details of our investigations with apples have appeared in a recent paper (Crane and Lawrence, 1930).

SUMMARY.

Sterility in fruits is of three kinds: (1) generational sterility; (2) morphological sterility; and (3) incompatibility. In the sweet cherry, *P. avium* (diploid), the behaviour of incompatibility is comparatively simple. Self-incompatibility is the rule, cross-incompatibility common and always reciprocally expressed. Among established varieties we have so far been able to recognise nine intra-sterile, inter-fertile groups. ·

A factorial analysis of preliminary investigations with seedlings shows that incompatibility in the sweet cherry is an orderly phenomenon and amenable to the genetic interpretation advanced by East and Mangelsdorf in *Nicotiana*, Lehmann in *Veronica* and, to some extent, to Sirks' interpretation in *Verbascum*. In the sour cherry (*P. cerasus*) and the Dukes (tetraploids) different degrees of compatibility occur.

In the plums, *P. domestica* (hexaploid), incompatibility is complex. Self- and cross-compatibility, self- and cross-incompatibility, degrees of self- and cross-incompatibility and differences in reciprocal matings, both complete and in partial degree, occur.

Cytologically, apples are of two kinds, (1) the secondarily balanced hexasomic tetraploids, and (2) the nonasomic hexaploids—the so-called "diploid" and "triploid" varieties respectively. The "triploids" are characterised by a high degree of generational sterility, but it is expressed in the formation of imperfect seeds and weak offspring rather than by failure to form fruits. In apples compatibility is common in partial degree, but rarely completely expressed.

The expression of incompatibility is more complex and variable in polyploids than in diploids. It is concluded that this is attributable to the polysomic condition of the factors which determine incompatibility in polyploids, and consequent interactions favourable to a greater variation in pollen-tube growth.

The more complex the polyploidy the less frequently will incompatibility occur, since the chances of individuals of similar constitution meeting to form incompatible groups are considerably reduced.

One way incompatibility is a phenomenon directly attributable to the multiplicity of factors in a polyploid, and we have suggested that

the frequency with which Sirks (1926 *a*) and others encountered such differences in reciprocal matings would indicate that the plants they investigated are polyploids.

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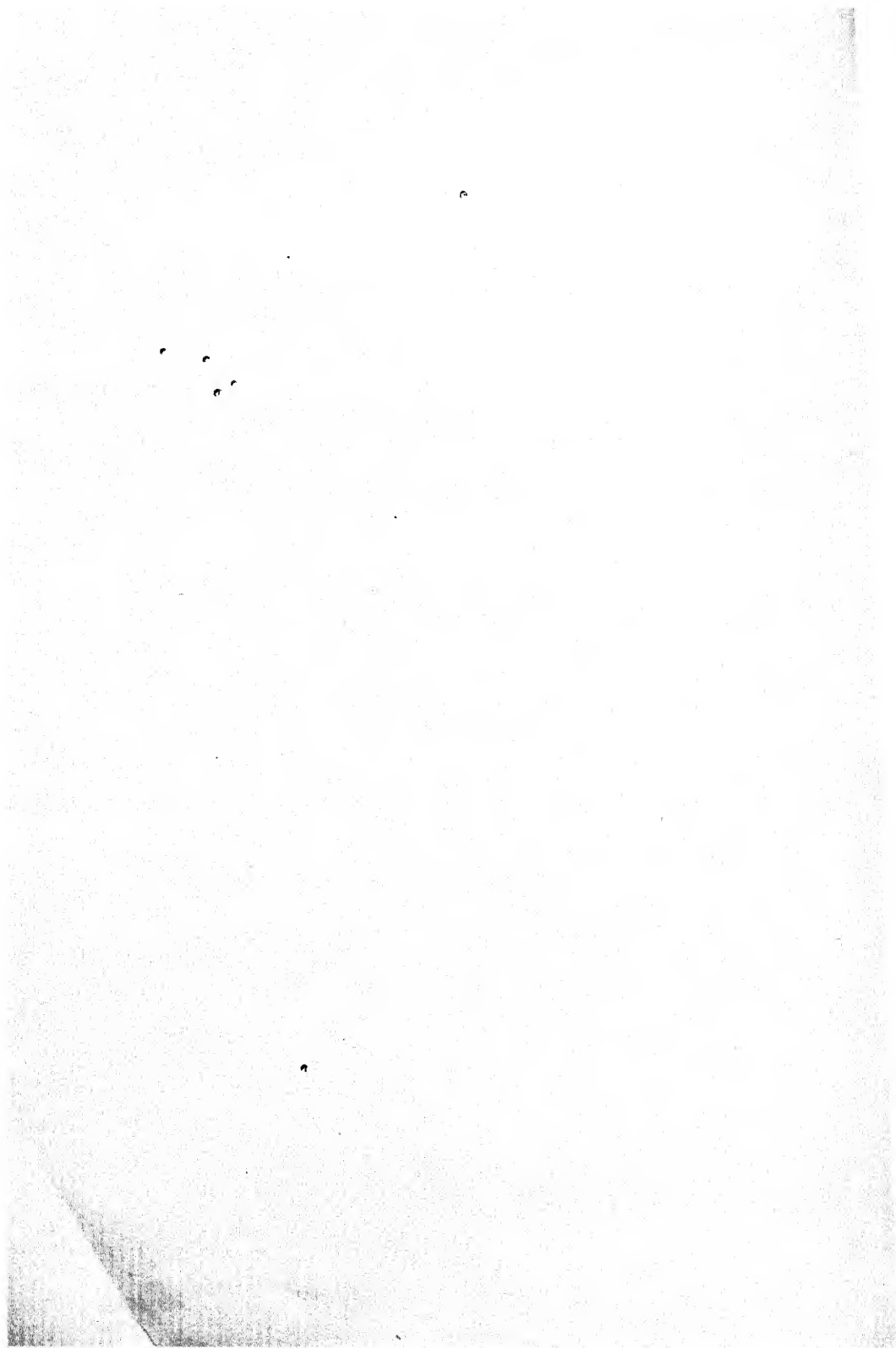
GENETICAL EXPERIMENTS WITH *SILENE OTITES* AND RELATED SPECIES.

BY THE LATE W. C. F. NEWTON.

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(With Plate II and One Text-figure.)

THIS paper contains the results of work performed by Mr Newton before his death; necessarily it contains no conclusive remarks upon the determination of sex in *Silene Otites*. The problem was of considerable interest to Mr Newton, and since the lines of the work are important, publication of the results is thought advisable. The first portion of the paper (up to p. 111) is taken from Mr Newton's notes, while the remaining portions are the result of collation of the records by Mrs L. Newton, Professor of Botany, University College, Aberystwyth, and F. W. Sansome.

Although an attempt has been made to interpret the facts along the lines of Mr Newton's presumed views the difficulties are obvious, and therefore the whole responsibility for the latter portion of the paper and the discussion, should fall on F. W. Sansome, who is continuing the work.

Silene Otites Otth. is a species which covers a wide geographical area, occurring in Europe, Siberia and western Asia as far as Persia. It is remarkable for the wide range of variation, and each type is limited to a part of the range of the aggregate species. A number of these types have now been separated by systematists, probably correctly, as distinct species. The plants are sub-dioecious, and Kerner among others reports male, female and hermaphrodite individuals in the wild populations. The female flowers show no trace of male organs, but a vestigial ovary is always present in the male flower. As a rule these vestigial ovaries contain no placentae or ovules, but, exceptionally, there appears a development of female characteristics—larger ovaries containing ovules being produced on some flowers of a male plant. This intersexual appearance is noticeably affected by the environment. At the end of the season, or after disturbance of the plant while it is in flower, the appearance of functional ovaries on normally male flowers is noticeable. In the cultures here dealt with, however, this tendency towards femaleness in the male plants has only occurred in the case of certain hybrids which will be dealt with below.

Large numbers of the British form of *S. Otites*, and also of the variety *umbellata* in the wild state, were examined, but no hermaphroditism was detected. Correns, among others, reports the presence of hermaphrodites in Germany. (It will be suggested later that in areas where two forms are inter-mixed the occurrence of a certain number of inter-sexual types might be expected. F. W. S.)

Briefly, the species contains pure females together with males which are of different grades of inter-sexuality. In view of the above considerations, it was decided to investigate the results of reciprocally crossing a number of forms of *Silene Otites* agg. with respect to the sex ratio and fertility of the progeny. The varieties and species used were as follows.

Silene Otites Otth., obtained from Barnham Common, Norfolk, is the smallest and most slender form of *Otites* that has been used. It was approached most closely by plants from the sand dunes of Jutland, kindly sent by C. A. Jørgensen.

S. Otites var. *umbellata* D.C., obtained from the sand dunes west of St Pierre, Quiberon (Morbihan), France, is a much stouter and more hairy plant than *S. Otites* Otth., and has a remarkably condensed inflorescence.

S. pseudotites Bess., obtained from the Italian Karst region near Briscike, north of Trieste, is coarser in habit and more viscid than the others.

S. wolgensis Roth., obtained from Bulgaria.

For these two latter species and for much information thanks are due to Dr W. B. Turrill of the Royal Botanic Gardens, Kew.

For the sake of brevity *S. Otites* from Norfolk will be referred to as *O*, var. *umbellata* as *U*, *pseudotites* as *P* and *wolgensis* as *W*.

The results of inter-crossing within three of the types are as follows:

$$O \times O \quad \text{gave } 38\ \male,\ 31\ \female;$$
$$P \times P \quad ,, \quad 37\ \male,\ 33\ \female;$$
$$U \times U \quad ,, \quad 11\ \male,\ 13\ \female.$$

For the inter-specific crosses between *O* and *P* two males and one female from *O* and one male and two females from *P* were used. Reciprocal crosses between the species were made in all possible ways.

In raising family 1/25 (*P* × *O*), in which the plants were covered in the field, it became evident that either the female was not covered at a sufficiently early stage, or that the muslin used was insufficiently fine; for besides the hybrids, which could be distinguished by their yellowish green colour and to some extent by their smaller size, there were a

number of non-hybrid plants of which nine were male and ten female, and further there were two hermaphrodites of which the origin is uncertain[1].

No sign of variegation has occurred in the families resulting from inter-crossing within the types themselves. The hybrid $P \times O$, however, is green in the cotyledonary stage but of yellower tinge than $P \times P$, while $O \times O$ in the same stage is a very dark green, owing to the presence of anthocyanin in the leaves. As the young plants of $(P \times O)$ develop, their young leaves are uniformly yellowish green, but they gradually turn green with age. This is also the case in the crosses $P \times U$ and $(P \times O) \times P$, in fact in all crosses where P has been used as the mother. Exceptionally (eight plants in all of the cross $P \times O$) yellow sectors which do not turn green are developed in certain leaves. In the reciprocal cross the young plants in the cotyledonary stage are white or yellowish white, and only occasional plants are variegated with green. The white seedlings die, and it is only from the comparatively rare variegated seedlings that a family can be raised. This explains the smallness of the families 1/26 and 2/25 which resulted from many hundred seeds. As the young plants develop, all are sectorially variegated with white or yellowish white, but in course of time the green parts become predominant. For example, in 1/26 there were 89 plants in March 1926, all of which were variegated, while of the 41 plants left in July 1927, 26 were quite green and 15 variegated.

Similar sectorial variegation has been observed in $U \times P$, $(P \times O) \times O$, $O \times (P \times O)$. Table I summarises the occurrence of variegation in various crosses.

Table II gives the results of the various crosses made. Where P was used as the female parent with O or U as male parent, there was a preponderance of females in the progeny, while the reciprocal crosses $(O \times P)$ gave a progeny with approximately normal sex ratio. The succeeding generations, however, have usually an excess of males together with an occasional hermaphrodite.

In the case of U, when used as a female parent in the cross $U \times P$, females are also in excess in the progeny.

The preponderance of females is great in the cross $P \times O$. No males, but two inter-sexes, appeared in 1/25, and four males and two inter-sexes made their appearance in the first year of growth in family 19/28. Males were not found in 5/26 in the first year of observation, but two males were found and were used as pollen parents in 1928. 11/27, the F_2 off-

[1] Presumably these inter-sexes were considered $(P \times O)$—see Table II, 11/27. F. W. S.

spring resulting from a selfing of an inter-sexual F_1 plant of the original cross $P \times O$, is interesting in that three of the six female plants carried hermaphrodite flowers on some of the later flowering axillary branches, while three males behaved similarly, making a total of eleven hermaphrodites. An illustration of a female inter-sex in this line appears in Text-fig. 1. Of the nine inter-sexes in 4/27 seven showed axes with hermaphrodite flowers which were weighted towards the female side in morphology. The two remaining inter-sexes were of the sub-androecious type found in other lines in these experiments.

The plants were scored as inter-sexes if any flowers on the raceme bore both pollen and fertilised ovules—except in the exceptional case of

TABLE I.

Variegation and sex ratio.

Cross	Family	No. at germination	No. variegated and type of variegation	Males	Females	Hermaphrodites
$O \times P$	2/25	Many	All. Albino and chlorina	1	1	—
	1/26	89	All. 37 chlorina, 15 mericlinal	22	29	—
$O \times U$	1/27	100	6 albino, 3 chlorina	51	49	—
$U \times O$	2/27	13	1 chlorina	10	3	—
$P \times U$	3/27	Over 300	All green	—	73	—
$U \times P$	4/27	128	33 mericlinal	20	87	9
$P \times O$	1/25	90	3 mericlinal	—	67	2
	4/26	13	12 chlorina, 1 mericlinal	—	13	—
	5/26	131	All chlorina, 4 mericlinal	—	119	—
	19/28	23	All chlorina	4	10	1 + 1?
$P \times (O \times W)$	21/28	105	34 chlorina	—	65	—
$(P \times O) \times O$	16/27	80	Some chlorina	22	17	—
	17/27	80	14 mericlinal	40	39	—
	18/27	80	Some chlorina	20	20	—
	19/27	80	Mostly chlorina, 16 mericlinal	30	22	—
	20/27	230	Few chlorina, 15 mericlinal	25	20	—

the female in 11/27 bearing hermaphrodite flowers, where the anthers were abortive and contained no pollen. In the majority of the inter-sexes, male flowers alone are produced on the oldest part of the raceme. Later in the season hermaphroditic flowers make their appearance, and are usually accompanied by a condensation of the internodes of the inflorescence. In addition to this, a few cases are known in which apparently female plants have produced one or more racemes either with inter-sexual or purely male flowers.

Table III sets out the results of the experiments involving an inter-sex as female parent. It will be seen that these differ considerably from analogous crosses made by Correns between *Silene Roemeri* and *S. Otites*,

and also differ from the results obtained in experiments with the dioecious species of *Melandrium*, described by the Hertwigs and by Shull. In the

TABLE II.

Summarised Results of the various crosses.

Cross	Family	Origin	No. of seedlings	Males	Females	Hermaph-rodites	Totals Males	Females	Hermaph-rodites
$O \times P$	2/25	01 × 03	2	1	1	—	23	30	0
	1/26	01 × 03	89	22	29	—			
$P \times O$	1/25	04 × 02	90	—	67	2	4	209	4
	4/26	04 × 06	13	—	13	—			
	5/26	04 × 06	131	—	119	—			
	19/28	P 4/27 × 2¹/26	23	4	10	2			
$(P \times O) \times P$	9/26	1⁵/25 × 03	22	9	12	—	27	40	1
	9/28	1⁵/25 × 6⁶/26	61	18	28	1			
$(P \times O) \times O$	7/26	1¹/25 × 02	18	—	18	—	235	202	0
	8/26	1³/25 × 02	73	17	19	—			
	14/27	1⁵/25 × 2¹/26	56	33	18	—			
	15/27	1¹⁰/25 × 2¹/26	—	18	6	—			
	16/27	1¹²/25 × 2¹/26	—	22	17	—			
	17/27	1¹³/25 × 2¹/26	83	40	39	—			
	18/27	5¹/26 × 2¹/26	—	20	20	—			
	19/27	5²/26 × 2¹/26	64	30	22	—			
	20/27	5³/26 × 2¹/26	52	25	20	—			
	21/27	5⁵/26 × 2¹/26	80	30	23	—			
$(O \times P) \times O$	10/27	2¹/25 × 2¹/26	45	17	16	—	31	29	2
	6/28	1³/26 × 2¹/26	130	14	13	1 + 1?			
$(O \times P) \times P$	7/28	2²/25 × 6⁶/26	161	35	31	2	35	31	2
$O \times (P \times O)$	8/28	2⁹/25 × 1¹/25	11	3	—	—	3	0	0
$P \times (O \times P)$	24/27	04 × 2¹/25	109	38	65	—	38	65	0
$(P \times O) \times (P \times O)$	11/27	1¹/25 selfed	69	41	6	5	*111	57	8
	25/27	1³/25 × 1¹/25	68	43	19	1			
	26/27	1¹²/25 × 1¹/25	67	27	32	2			
$(O \times P) \times (O \times P)$	9/27	2²/25 × 2¹/25	66	40	16	—	110	43	0
	28/27	1⁵/26 × 2¹/25	—	43	18	—			
	5/28	1²/26 × 1¹/26	373	27	9	—			
$O \times [(P \times O) \times O]$	1/28	2⁹/26 × 8²/26	—	17	17	—	17	17	0
$O \times U$	1/27	2¹/26 × 05	100	51	49	—	51	49	0
$U \times O$	2/27	05 × 2¹/26	14	10	3	—	87	107	6
	27ᵃ/27	05 × 2¹/26	—	37	55	6			
	27ᵇ/27	05 × 2¹/26	—	40	49	—			
$(O \times U) \times (O \times U)$	2/28	1¹/27 × 1²/27	85	20	27	—	20	27	0
$P \times U$	3/27	6¹/26 × 05	77	—	73	—	0	73	0
$U \times P$	4/27	05 × 6¹/26	128	20	87	9	20	87	9
$P \times (U \times P)$	20/28	P 4/27 × 4¹/27	2	—	1	—	0	1	0
$(U \times P) \times (U \times P)$	12/28	4²/27 × 4¹/27	86	3	4	—	5	5	0
	22/28	4⁵/27 selfed	—	2	1	—			
$(P \times U) \times (U \times P)$	13/28	3¹/27 × 4¹/27	319	69	24	1	69	24	1
$O \times W$	5/27	2/26 × K 100	—	29	28	—	29	28	0
$W \times O$	6/27	K 100 × 2¹/26	100	3	2	—	3	2	0
$W \times U$	7/27	K 100 × 05	100	6	6	—	6	6	0
$P \times (O \times W)$	21/28	P 4¹/27 × 5¹/27	105	—	65	—	0	65	0

* See text.

case of the family 11/27 (the result of selfing an inter-sex), females were obtained in about equal proportions to the inter-sexes present, together

with a preponderance of males. In two other lines females have also been noticed—11/26 and 3/28. Correns found no females in thousands of individuals arising from the selfing of an inter-sex in *S. Roemeri* or *S. Otites*.

As with Correns' results we find that the cross (female × inter-sex) produces males and females, but very few inter-sexes are present. See Table IV.

Table V indicates that when pollen from one male parent is used on different females of similar origin, the sex ratio of the progeny is similar. The sex ratio of the progeny remains the same so long as the female parents are of similar origin. When females of different origin were used, the sex ratios of the resulting progeny were often different.

Sex determination in *S. Otites* is influenced by both male and female parents. This is confirmed by Table VI which indicates that with one female, or two of similar constitution, different males give different sex ratios.

Table I illustrates the fact that sex ratios and survival percentage are not closely related, and that variegation has not a great effect on sex ratio. Variegation causing mortality does not appear to have a selective effect on the sex ratio in *S. Otites* agg., but there is a strong possibility that the abnormal sex ratio and variegation are resulting from one cause—the derangement of genic balance which is brought about by hybridity.

Text-fig. 1. Female inter-sex in F_2 of *S. pseudotites* × *S. Otites*.

The determination of variegation may be similar in kind to that described by Renner (1922, 1924) in *Oenothera*. *Oe. Lamarkiana* × *Oe. Hookeri* gives progeny with pale green foliage and these sometimes die.

TABLE III.

Hermaphrodites used as female parent.

Cross	Family	Origin	Males	Females	Hermaphrodites
$(P \times O)$ nat. seed.	11/26	Nat. seed.	6	1	1
$(P \times O) \times (P \times O)$	11/27	$1^1/25$ selfed	41*	6*	5*
$(U \times O) \times (U \times O)$	3/28	$27^3/27$ selfed	2	1	—
$(U \times O)$ nat. seed.	4/28	$27^3/27$ nat. seed.	66	—	13

* Three of the six females bore abortive stamens in some flowers, and three of the forty-one males later bore a few capsules.

TABLE IV.

Females × Hermaphrodite.

Cross	Family	Origin	Males	Females	Hermaphrodites
$(P \times O) \times (P \times O)$	13/27	$2^2/25 \times 1^1/25$	10	5	1
	25/27	$13/25 \times 1^1/25$	43	19	1
	26/27	$1^{12}/25 \times 1^1/25$	27	32	2
$O \times (O \times P)$	8/28	$2^9/25 \times 1^1/25$	3	—	—
	10/28	$26^1/27 \times 11^7/27$	33	35	—
	11/28	$26^1/27 \times 11^9/27$	32	17	—
$(U \times P) \times (U \times P)$	22/28	$4^5/27$ selfed	20	28	6

TABLE V.

The progeny of crosses using one male parent.

Cross	Family	Origin	Males	Females	Hermaphrodites	Totals Males	Totals Females	Totals Hermaphrodites
$P \times O$	19/28	P $4/27 \times 2^1/26$	4	10	2	4	10	2
$(O \times P) \times O$	10/27	$2^1/25 \times 2^1/26$	17	16	$1+1?$	31	29	2
	6/28	$1^5/26 \times 2^1/26$	14	13				
$(P \times O) \times O$	14/27	$1^5/25 \times 2^1/26$	33	18	—			
	15/27	$1^{10}/25 \times 2^1/26$	18	6	—			
	16/27	$1^{12}/25 \times 2^1/26$	22	17	—			
	17/27	$1^{15}/25 \times 2^1/26$	40	39	—	218	165	0
	18/27	$5^1/26 \times 2^1/26$	20	20	—			
	19/27	$5^2/26 \times 2^1/26$	30	22	—			
	20/27	$5^3/26 \times 2^1/26$	25	20	—			
	21/27	$5^5/26 \times 2^1/26$	30	23	—			
$U \times O$	2/27	$05 \times 2^1/26$	10	3				
	$27^a/27$	$05 \times 2^1/26$	37	55	6	87	107	6
	$27^b/27$	$05 \times 2^1/26$	40	49	—			
$W \times O$	6/27	K $100 \times 2^1/26$	3	2	—	3	2	0
$UP \times UP$	12/28	$4^2/27 \times 4^1/27$	3	4	—	3	4	0
$PU \times UP$	13/28	$3^1/27 \times 4^1/27$	69	24	1	69	24	1
$OP \times OP$	9/27	$2^2/25 \times 2^1/25$	40	16	—	40	16	0
$PO \times OP$	28/27	$1^5/26 \times 2^1/25$	43	18	—	43	18	0
$P \times OP$	24/27	$04 \times 2^1/25$	38	65	—	38	65	0

Some of the seedlings have dark green flecks on the first leaves. These can live and give rise to chimerical plants. The reciprocal hybrid is almost completely green, and individuals with light flecks on the first leaves are only seldom found. Similarly *Oe. suaveolens* × *Oe. muricata* gives rise to white seedlings and green-white chimaeras. The reciprocal cross, which is rarer in occurrence, is green, spotted with white to a certain extent.

TABLE VI.

The progeny of crosses using similar females.

Cross	Family	Origin	Males	Females	Hermaph-rodites
$(O \times P) \times P$	7/28	$2^2/25 \times 6^6/26$	35	31	2
$(O \times P) \times (O \times P)$	9/27	$2^2/25 \times 2^1/25$	40	16	—
$(O \times P) \times (P \times O)$	13/27	$2^2/25 \times 1^1/25$	10	5	1
$(O \times P) \times O$	10/27	$2^1/25 \times 2^1/26$	17	16	—
$(P \times O) \times O$	7/26	$1^1/25 \times O2$	—	18	—
	8/26	$1^3/25 \times O2$	17	19	—
$(P \times O) \times O$	14/27	$1^5/25 \times 2^1/26$	33	18	—
	15/27	$1^{10}/25 \times 2^1/26$	18	16	—
$(P \times O) \times P$	9/28	$1^5/25 \times 6^6/26$	18	28	1
$(P \times O) \times (P \times O)$	25/27	$1^3/25 \times 1^1/25$	43	19	1
	26/27	$1^{12}/25 \times 1^1/25$	27	32	2

DISCUSSION.

Mr Newton's investigation was unfortunately not completed, therefore the discussion must be purely tentative.

The important fact stands out that the sex ratios of the progeny of reciprocal crosses of *S. pseudotites* and *S. Otites* are different and that the succeeding generations may have abnormal sex ratios. A subsidiary result is the appearance of inter-sexes in some of the hybrid lines, while none have been found when the species have been inbred.

In *Melandrium album* and *M. rubrum*, dioecious species, the sexes are well marked, and under normal conditions the appearance of well-developed organs of one sex upon plants of the alternative sex type is rare. Further, genetical evidence (Shull, Hertwig, Correns, Winge) and cytological evidence (Blackburn, Winge, Meurman) show that, as in most animal groups, there is a heterochromosome pair in the male sex.

In *S. Otites* Blackburn suggests that there is a morphological difference visible at early anaphase, similar in kind but less in degree than that found in *Melandrium*, between a chromosome pair in the male. Newton's notes state: "I have not been able to distinguish a size difference." Dr Schatz, working with Correns, has been unable to discover a heterochromosome pair in a closely related species, *S. Roemeri*; therefore it is difficult to determine which sex in *S. Otites* is heterogametic. Haldane has

enunciated the rule that when one sex is absent, rare or sterile in the F_1 hybrid of two animal races, that sex is the heterogametic one. The cross $P \times O$, if the conditions in *Silene* bear any relation to those of animals, would indicate that the male was heterogametic. Correns concludes from his work on *S. Otites* and *S. Roemeri* that the male is heterogametic and the female homogametic for sex determination, but that many more independent factors are present which, if they enter into the male, cause a greater or less development of inter-sexuality. Correns found that when certain males were used with various females only female offspring were obtained. This thelygeny he believes to be analogous to that in *Melandrium*, where the hypothesis of a lethal factor present in the Y-chromosome will explain the facts.

Correns found no females from selfing or inter-crossing the male inter-sexes of *S. Otites*, but Newton found a small proportion of females. It is questionable whether more females would have arisen in Newton's experiments in the absence of the complications due to the hybrid nature of the plants. One expects that if the male is heterogametic the male inter-sexes would segregate into males, females and inter-sexes in a fashion analogous to the behaviour of *Melandrium* and several animals.

Correns and Newton show that females as well as males influence the sex ratio of the progeny. Newton's results further indicate that the parentage of the individuals used for crossing is of importance in determining the sex ratio of their progeny. The sex ratio of the progeny depends upon the amounts of P, O, U and W in the pedigree. It is interesting in this connection to note that the Karst region where P was collected has a population of *pseudotites* consisting mainly of inter-sexes, while not one has been found in the *Otites* population in Britain; but *Otites* from Langenstein does contain inter-sexual types. The great majority of inter-sexes occur in our lines where P enters the pedigree, hence it is tentatively suggested that some of the "independent factors" of Correns may be traced to hybrid origin, and in our lines to P.

This last fact may bear some analogy to the hybrids in *Bistoninae* (Harrison). When *Nyssia zonaria* was crossed with male *Poecilopsis pomonaria*, obtained from the wild, only males appeared in the progeny, but if *P. pomonaria* was inbred for several generations and then used as male on *Nyssia* seven females in seventy-seven individuals were found. Harrison suggests that by inbreeding *P. pomonaria* the male potencies are reduced, and so the WZ mechanism of *Nyssia* is more able to influence the characters than when crossed with the strong male-determining qualities of the wild *P. pomonaria*. The isolation of *S. Otites* in Britain may have

considerably reduced the male potency in comparison with the potency of the female determiners of P. When crossed with a P these male determiners are restored in the F_1 and the swing to the female side is stopped on further crossing with this F_1. As with the *Bistoninae* hybrids the reciprocal cross ($O \times P$) gives a normal sex ratio.

This also suggests analogy with experiments on *Lymantria dispar* and *L. japonica* (Goldschmidt). Different males with similar females give different results and similar males with different females give different results. There is, however, dissimilarity between the results in *Silene* and *Lymantria*. Whereas in *Lymantria* an F_1 with strong female tendencies produces an F_2 with female tendencies, the sex ratio of the F_2 of ($P \times O$) in *Otites* gives an excess of males and no signs of female prepotency! The cross ($P \times O$) $\times O$ was made a number of times by Mr Newton in order to test the genetic constitution of ($P \times O$) in regard to sex. The results are not entirely conclusive. One hybrid 14/27 has an excess of males while the other crosses approach equality of males and females, but there is still a bias towards maleness. Nevertheless, the evidence indicates that few if any of the F_1 females of ($P \times O$) are genetic males.

In certain respects the sex determination of *S. Otites* bears a resemblance to that of polyoecious species such as *Mercurialis annua* (Yampolsky), *Plantago lanceolata* and *Valeriana dioica* (Correns). These species have several grades of inter-sexes, each of which tends to reproduce itself. If two inter-sexes of different grades are crossed, the mean grade of inter-sexuality of the progeny is between that of the parents, and the range of grades is greater from such a cross than from either of the parents selfed. The suggestion has been made that there is a valency difference in sex determining potency of the gametes furnished by the different individuals, such that the stronger the sex expression of an individual for one type of sexuality the stronger will be its effect on the progeny. But if any scheme is to be accepted as valid it must account for the non-segregation of the types when inbred, and so far none has been found.

SUMMARY.

1. The investigation concerns *Silene Otites* Otth., *S. Otites* var. *umbellata* D.C., and the related species *S. pseudotites* Bess. and *S. wolgensis* Roth. These plants were obtained from various localities in Europe.

2. Within any one of the species the sex ratio is normal.

3. The cross between *pseudotites* as female and *Otites* or *umbellata* as male gives a large preponderance of females, while the reciprocal cross gives approximately equal numbers of male and female in the first

generation. The (F_2) generation of these crosses also exhibits abnormalities in the sex ratio and in sex expression.

4. No inter-sexes have been seen among the British wild forms or among the progeny resulting from inbred lines of any of the subspecies concerned. When inter-crossed, however, a small proportion of inter-sexes occurs in some families.

5. As a result of crossing one female with different males and also several females with one male it is clear that both parents influence the sex ratio of the progeny.

6. Variegation occurs when *pseudotites* is used as male or female parent in a specific cross, but no variegation has appeared when the species have been inbred. There is no apparent influence of variegation upon the sex ratio (primary and secondary sex ratios cannot be discriminated).

7. Inter-sexes, when used as the female parent, have thrown a proportion of females together with a majority of males and a small percentage of inter-sexes. This is contrary to the results of Correns with closely related species.

8. Females crossed with inter-sexes give rise to females and males in approximately equal proportions, together with a small percentage of inter-sexes. The number of inter-sexes in the progeny is little influenced by the grade of inter-sexuality of the pollen-bearing parent.

9. No hypothesis has been found which completely meets the case. The difference of sex ratios of reciprocal crosses is not greatly influenced by cytoplasmic effects, nor is the adoption of a theory of a valency gradient of sex potencies at present feasible.

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EXPLANATION OF PLATE II.

Fig. 1. Female plant of *S. Otites* Otth.
Fig. 2. Male of same.
Fig. 3. Female of *S. Otites* var. *umbellata* D.C.
Fig. 4. Male of same.
Fig. 5. Male of *S. pseudotites* Bess.
Fig. 6. Female of same.

PLATE II

LINKAGE IN THE TETRAPLOID *PRIMULA SINENSIS.*

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CONTENTS.

INTRODUCTION.

LINKAGE has so far been almost exclusively studied in diploids and allopolyploids such as wheat. In the latter the phenomenon is not essentially different from that in diploids, as each chromosome, save for rare exceptions, has a definite mate. The only case of true polyploid linkage so far studied is that in the triplo-**X** *Drosophila melanogaster* of Bridges and Anderson (1925). No linked factors were known in auto-tetraploid plants other than *Primula sinensis* until 1930, but our colleague Dr Sansome is now studying linkage in tetraploid tomatoes.

The present work was begun in 1909 by the late R. P. Gregory. Sverdrup Sömme (1930) has analysed the data up to 1927. We incorporate counts of 2867 more plants, but reject some of her data on various grounds.

The factors here considered are **S**, **B** and **G**. **S** converts a pin plant, with long style and short stamens, into a thrum with short style and long stamens. **B** converts red flower pigment into magenta, **G** inhibits the formation of anthocyanin pigment in the centre of the flower, producing a green stigma and ovary in place of a red. In the diploid they are completely dominant. In the tetraploid **S** is completely so, but **Bbbb** and **Gggg**, though generally easily distinguished from **bbbb** and **gggg**, are on the whole not so different from them as are **BBBB** and **GGGG**. There is

no possibility of a mistake in scoring G. In some families **Bbbb** and **bbbb** may have been confused. It is possible, though not very likely, that errors may have been made regarding **G**. No attempt has been made to separate different grades of dominant, *e.g.* **BBbb** and **Bbbb**, though in some cases this was partly possible.

The linkage values of these factors in the diploid are based on a mass of material, partly given by Gregory, de Winton and Bateson (1923). Table I is based entirely on back-cross data as regards the diploid; the F_2 data are concordant, but do not enable a distinction to be made between the male and female sides of the plant. The data on which the tetraploid figures are based will be given later.

TABLE I.

Cross-over values per cent., with standard errors.

Factors	Diploid ♀	Diploid ♂	Tetraploid ♀	Tetraploid ♂
SB	7·35 ± 0·40	12·91 ± 0·50	8·01 ± 0·69	8·41 ± 0·99
SG	33·29 ± 0·74	40·47 ± 0·78	37·58 ± 1·92	38·91 ± 2·23
BG	31·15 ± 0·53	36·24 ± 0·68	35·18 ± 1·85	34·38 ± 2·17

In order to understand the linkage data we must first consider the genetical behaviour of the factors one at a time. Apart from aneuploids, *e.g.* plants with $4n + 1$ (49) chromosomes, the following types of zygote are to be expected with regard to a single pair of allelomorphs **X** and **x**: **XXXX**, quadruplex; **XXXx**, triplex; **XXxx**, duplex; **Xxxx**, simplex; **xxxx**, nulliplex.

Quadruplex and triplex plants give no recessive offspring, duplex by nulliplex give 5 dominant : 1 recessive, simplex by nulliplex 1 dominant : 1 recessive. In Table II actual figures are given for the factors concerned. These figures do not represent all the material available, but only those in which the composition of the dominant parent was known from its ancestry. All the duplex plants included in them were from the cross **XXXX** × **xxxx** or reciprocal. The only element of doubt here is the possibility of an alleged **XXXX** grand-parent having been **XXXx**, such triplex plants being indistinguishable from quadruplex by a single generation of breeding, and being eliminated rather slowly on self-fertilisation. Plants known by their genetic behaviour, but not their ancestry, to have been **XXxx** are excluded. Such plants occurred, for example, among the progeny when **XXxx** was selfed.

Similarly the only **Xxxx** plants whose progeny is included are those from **Xxxx** × **xxxx** or the reciprocal cross, the constitution of the simplex grand-parent being assumed, if necessary, from its genetical behaviour. Dominant progeny of 73 crosses of simplex × nulliplex have been tested,

and all have proved simplex. On the hypothesis of random assortment of chromatids (Haldane, 1930) one in thirteen would have been duplex.

TABLE II.

Single factor ratios.

Parents	No. of families	Dominant	Recessive	D	$D \div \sigma$
$Ss_3 \times Ss_3$	40	1282	395	−24·25	1·20
$Bb_3 \times Bb_3$	17	358	124	+ 3·5	0·37
$Gg_3 \times Gg_3$	10	265	91	+ 2	0·24
$Ss_3 \times s_4$	75	1001	1009	+ 4	0·18
$Bb_3 \times b_4$	38	559	542	− 8·5	0·51
$Gg_3 \times g_4$	25	368	371	+ 1·5	0·11
$s_4 \times Ss_3$	45	456	534	+39	2·48
$b_4 \times Bb_3$	28	291	380	+44·5	3·43
$g_4 \times Gg_3$	24	293	315	+11	0·89
$S_2s_2 \times S_2s_2$	1	44	2	+ 0·7	0·65
$G_2g_2 \times G_2g_2$	23	1204	44	+10	1·74
$G_2g_2 \times g_4$	34	643	125	− 3	0·29
$g_4 \times G_2g_2$	9	87	22	+ 3·8	0·98

Table II shows the genetical behaviour of simplex and duplex plants. The results are not independent, owing to linkage. It will be seen that the only really serious deviations from expectation occur in the cross of $x_4 \times Xx_3$, the heterozygotes giving an excess of recessive gametes on the male side. The fact that the same families are included in the **B** and **S** totals accounts for the similar discrepancy in both cases, since **B** and **S** were coupled in many of the plants. Five **Bb₃** plants as males gave 66 **B**, 106 **b**, which accounts for nearly half the discrepancy. Used as females they gave 90 **B**, 85 **b**. We have clearly to deal with a case of anisogeny, the **B** pollen grains being handicapped while the **B** ovules are not. A possible explanation is that these plants were **Bbbbb**, *i.e.* 49 chromosome plants, and that as in *Datura* $2n + 1$ ovules are functional, $2n + 1$ pollen not so in competition with $2n$ pollen. In this case the ovules would give a ratio of 1 **B** : 1 **b**, the pollen grains 2 **B** : 3 **b**, which agrees with observation. If the divergence from expectation were due to random pairing of chromatids we should expect similar gametic ratios on both sides, for the equality of linkage values suggests that meiosis is similar on the two sides of the plant. If non-disjunction of the **SBG** chromosome is at all common we should expect to find **Bbbbb** plants among the parents of Table II; on the other hand **Bbbb** × **bbbb** or the reciprocal cross could not give **BBbbb** apart from double reduction.

Exceptions due to double reduction may occur, but they are too rare to be considered in an admittedly preliminary theory of linkage.

Hence in what follows we assume that two chromatids from the same chromosome always go into different gametes. This is borne out by the behaviour of linked factors. The further question whether two chromatids which have paired and exchanged factors by crossing-over may enter the same gamete is considered later. Both these anomalies would involve non-disjunction.

THEORY OF LINKAGE IN TETRAPLOIDS.

In a diploid organism heterozygous for two factors we can only study two types of gametic series, those characteristic of coupling and repulsion, which are closely related, and their relation could be deduced on many different theories of segregation. For example, it was consistent with the reduplication theory, or with several different theories as to the relation between factors and chromosomes.

In the tetraploid, however, there should be seven distinct types of gametic series: (1) single coupling, (2) single repulsion, (3) asymmetrical coupling, (4) asymmetrical repulsion, (5) double coupling, (6) double repulsion, (7) coupling *and* repulsion. It will be difficult to construct zygotes giving combined coupling and repulsion, or to identify them with certainty if they have been constructed.

We consider only the ratios to be expected in the case of completely dominant factors. In order to obtain visible segregation the number of these factors in a zygote must be one or two. Suppose two factors **X** and **Y** to be linked, crossing-over occurring in the formation of a proportion p of the gametes; and a plant known from its genetical performance to be of the composition

$$XY$$
$$xy$$
$$xy$$
$$xy$$

Then if, after crossing-over has occurred, two chromosomes can enter the same gamete, some of its **XY** offspring when it is crossed with $(xy)_4$ will exhibit repulsion of **X** and **Y**. As will be seen later, such an event is rare, if it occurs at all. In what follows we will assume that it does not occur, *i.e.* that after two chromosomes have paired, they must proceed to different poles. The absence of such a conversion of coupling into repulsion also renders pairing of three chromosomes leading to "progressive" crossing-over unlikely, and further reasons are given later to show that it is a rare or non-existent phenomenon.

Case 1. Single coupling.

$$XY$$
$$xy$$
$$xy$$
$$xy$$

The chromosome containing **X** and **Y** must pair with one of the others. Crossing-over occurs in p of the total cases, the gametic series is therefore:

$$(1-p) \; \mathbf{XY} : p \; \mathbf{Xy} : p \; \mathbf{xY} : (1-p) \; \mathbf{xy}$$
$$ \mathbf{xy} \mathbf{xy} \mathbf{xy} \mathbf{xy}$$

a series similar to that of the diploid.

Case 2. Single repulsion.

$$\mathbf{Xy}$$
$$\mathbf{xY}$$
$$\mathbf{xy}$$
$$\mathbf{xy}$$

Calling the four chromosomes A, B, C and D, in two-thirds of all cases A does not pair with B, so the gametes are:

$$1 \; \mathbf{Xy} : 1 \; \mathbf{Xy} : 1 \; \mathbf{xY} : 1 \; \mathbf{xy}$$
$$ \mathbf{xY} \mathbf{xy} \mathbf{xy} \mathbf{xy}$$

In the remaining one-third A and B pair, so there is a chance of crossing-over, the gametes being:

$$p \; \mathbf{XY} : (1-p) \; \mathbf{Xy} : (1-p) \; \mathbf{xY} : p \; \mathbf{xy}$$
$$ \mathbf{xy} \mathbf{xy} \mathbf{xy} \mathbf{xy}$$

Hence the total gametic series is:

$$1 \; \mathbf{Xy} : p \; \mathbf{XY} : (2-p) \; \mathbf{Xy} : (2-p) \; \mathbf{xY} : (1+p) \; \mathbf{xy}$$
$$ \mathbf{xY} \mathbf{xy} \mathbf{xy} \mathbf{xy} \mathbf{xy}$$

Case 3. Asymmetrical coupling.

$$\mathbf{XY}$$
$$\mathbf{Xy}$$
$$\mathbf{xy}$$
$$\mathbf{xy}$$

Here in one-third of all cases AB, CD pair and the gametes are:

$$1 \; \mathbf{XY} : 1 \; \mathbf{Xy}$$
$$ \mathbf{xy} \mathbf{xy}$$

In the other cases crossing-over may occur between A and C or D, and the resulting chromosome has an equal chance of entering an **Xy** or an **xy** gamete. The total gametic series is therefore:

$$(1-p) \; \mathbf{XY} : (2-p) \; \mathbf{XY} : p \; \mathbf{Xy} : p \; \mathbf{Xy} : 2 \; \mathbf{Xy} : p \; \mathbf{xY} : (1-p) \; \mathbf{xy}$$
$$ \mathbf{Xy} \mathbf{xy} \mathbf{xY} \mathbf{Xy} \mathbf{xy} \mathbf{xy} \mathbf{xy}$$

Case 4. Asymmetrical repulsion.

$$\begin{matrix} Xy \\ Xy \\ xY \\ xy \end{matrix}$$

In one-third of all cases AB, CD pair and the gametes are:

$$\begin{matrix} 1\ Xy : 1\ Xy \\ xY \quad\quad xy \end{matrix}$$

In the other two-thirds pairing of C with A or B renders crossing-over possible, the resulting chromosomes as above entering a gamete with **Xy** or **xy** in equal numbers. The total gametic series is thus:

$$p\ \begin{matrix}XY \\ Xy\end{matrix} : p\ \begin{matrix}XY \\ xy\end{matrix} : (2-p)\ \begin{matrix}Xy \\ xY\end{matrix} : (1-p)\ \begin{matrix}Xy \\ Xy\end{matrix}\ \begin{matrix} \\ xy\end{matrix} : 2\ \begin{matrix}Xy \\ xy\end{matrix} : (1-p)\ \begin{matrix}xY \\ xy\end{matrix} : p\ \begin{matrix}xy \\ xy\end{matrix}$$

Case 5. Double coupling.

$$\begin{matrix} XY \\ XY \\ xy \\ xy \end{matrix}$$

In one-third of all cases AB, CD pair and the gametes are $\begin{smallmatrix}XY \\ xy\end{smallmatrix}$. In two-thirds of the cases crossing-over may occur. The following question now arises. Does the fact that crossing-over has occurred between chromosomes A and C alter the probability of crossing-over between B and D? This probability would be increased if certain variable conditions in the nucleus as a whole favoured crossing-over in both cases. It would be diminished if, for example, only a finite amount of energy was available for twisting or breaking the chromosomes, and this might be concentrated on one pair or the other. In what follows we shall assume that the probabilities are independent, an hypothesis which agrees fairly well with experience. The gametic output where crossing-over is possible is therefore symbolised by

$$[(1-p)\ XY : p\ Xy : p\ xY : (1-p)\ xy]^2$$

and the total gametic series is:

$$(1-2p+p^2)\ \begin{matrix}XY \\ XY\end{matrix} : (2p-2p^2)\ \begin{matrix}XY \\ Xy\end{matrix} : (2p-2p^2)\ \begin{matrix}XY \\ xY\end{matrix} :$$

$$2\ (2-2p+p^2)\ \begin{matrix}XY \\ xy\end{matrix} : 2p^2\ \begin{matrix}Xy \\ xY\end{matrix} : p^2\ \begin{matrix}Xy \\ Xy\end{matrix} : (2p-2p^2)\ \begin{matrix}Xy \\ xy\end{matrix} :$$

$$p^2\ \begin{matrix}xY \\ xY\end{matrix} : (2p-p^2)\ \begin{matrix}xY \\ xy\end{matrix} : (1-p)^2\ \begin{matrix}xy \\ xy\end{matrix}$$

In the event of a positive or negative correlation between the two cross-overs the terms whose coefficients are divisible by p would be increased or diminished respectively.

Case 6. Double repulsion.

$$\begin{array}{c} \mathbf{Xy} \\ \mathbf{Xy} \\ \mathbf{xY} \\ \mathbf{xY} \end{array}$$

In one-third of all cases AB, CD pair and the gametes are all $\begin{array}{c}\mathbf{Xy}\\\mathbf{xY}\end{array}$. In the remaining two-thirds crossing-over may occur. If it is independent the output is symbolised by

$$[p\ \mathbf{XY} : (1-p)\ \mathbf{Xy} : (1-p)\ \mathbf{xY} : p\ \mathbf{xy}]^2$$

and the total gametic series is:

$$p^2\ \begin{array}{c}\mathbf{XY}\\\mathbf{XY}\end{array} : (2p-2p^2)\ \begin{array}{c}\mathbf{XY}\\\mathbf{Xy}\end{array} : (2p-2p^2)\ \begin{array}{c}\mathbf{XY}\\\mathbf{xY}\end{array} : 2p^2\ \begin{array}{c}\mathbf{XY}\\\mathbf{xy}\end{array} : (4-4p+2p^2)\ \begin{array}{c}\mathbf{Xy}\\\mathbf{xY}\end{array} :$$

$$(1-p)^2\ \begin{array}{c}\mathbf{Xy}\\\mathbf{Xy}\end{array} : (2p-2p^2)\ \begin{array}{c}\mathbf{Xy}\\\mathbf{xy}\end{array} : (1-p)^2\ \begin{array}{c}\mathbf{xY}\\\mathbf{xY}\end{array} : (2p-2p^2)\ \begin{array}{c}\mathbf{xY}\\\mathbf{xy}\end{array} : p^2\ \begin{array}{c}\mathbf{xy}\\\mathbf{xy}\end{array}$$

subject to the above reservation.

Case 7. Coupling and repulsion.

$$\begin{array}{c} \mathbf{XY} \\ \mathbf{Xy} \\ \mathbf{xY} \\ \mathbf{xy} \end{array}$$

In one-third of all cases AB, CD pair and the gametes are:

$$1\ \begin{array}{c}\mathbf{XY}\\\mathbf{xY}\end{array} : 1\ \begin{array}{c}\mathbf{XY}\\\mathbf{xy}\end{array} : 1\ \begin{array}{c}\mathbf{Xy}\\\mathbf{xY}\end{array} : 1\ \begin{array}{c}\mathbf{Xy}\\\mathbf{xy}\end{array}$$

In one-third of all cases AC, BD pair and the gametes are:

$$1\ \begin{array}{c}\mathbf{XY}\\\mathbf{Xy}\end{array} : 1\ \begin{array}{c}\mathbf{XY}\\\mathbf{xy}\end{array} : 1\ \begin{array}{c}\mathbf{Xy}\\\mathbf{xY}\end{array} : 1\ \begin{array}{c}\mathbf{xY}\\\mathbf{xy}\end{array}$$

In one-third of all cases AD, CB pair and in the absence of correlation the gametic series is represented by

$$[p\ \mathbf{XY} : (1-p)\ \mathbf{Xy} : (1-p)\ \mathbf{xY} : p\ \mathbf{xy}]$$
$$\times\ [(1-p)\ \mathbf{XY} : p\ \mathbf{Xy} : p\ \mathbf{xY} : (1-p)\ \mathbf{xy}]$$

Hence the total gametic series is:

$$(p - p^2)\ \frac{\mathbf{XY}}{\mathbf{XY}} : (2 - 2p + 2p^2)\ \frac{\mathbf{XY}}{\mathbf{Xy}} : (2 - 2p + 2p^2)\ \frac{\mathbf{XY}}{\mathbf{xY}} :$$

$$(2 + 2p - 2p^2)\ \frac{\mathbf{XY}}{\mathbf{xy}} : (2 + 2p - 2p^2)\ \frac{\mathbf{Xy}}{\mathbf{xY}} : (p - p^2)\ \frac{\mathbf{Xy}}{\mathbf{Xy}} :$$

$$(2 - 2p + 2p^2)\ \frac{\mathbf{Xy}}{\mathbf{xy}} : (p - p^2)\ \frac{\mathbf{xY}}{\mathbf{xY}} : (2 - 2p + 2p^2)\ \frac{\mathbf{xY}}{\mathbf{xy}} : (p - p^2)\ \frac{\mathbf{xy}}{\mathbf{xy}}$$

provided that, in the third case, crossing-over is not correlated.

Since in the case of complete dominance, the various classes of gamete containing at least one **X** and **Y** produce indistinguishable zygotes, the above results may be summarised in Table III. p and q are the cross-over ratios on the two sexual sides of the plant.

TABLE III.

Type of zygote	Types of gametes	Gametes in general	Gametes, $p=0$	Gametes, $p=\frac{1}{2}$	Gametes in absence of linkage	Zygotic ratio on selfing
1. $\mathbf{XY}.(\mathbf{xy})_3$	XY	$1-p$	1	1	1	$2+(1-p)(1-q)$
	Xy	p	0	1	1	$1-(1-p)(1-q)$
	xY	p	0	1	1	$1-(1-p)(1-q)$
	xy	$1-p$	1	1	1	$(1-p)(1-q)$
2. $\mathbf{Xy}.\mathbf{xY}.(\mathbf{xy})_2$	XY	$1+p$	1	1	1	$18+(1+p)(1+q)$
	Xy	$2-p$	2	1	1	$9-(1+p)(1+q)$
	xY	$2-p$	2	1	1	$9-(1+p)(1+q)$
	xy	$1+p$	1	1	1	$(1+p)(1+q)$
3. $\mathbf{XY}.\mathbf{Xy}.(\mathbf{xy})_2$	XY	$3-p$	3	5	5	$26+(1-p)(1-q)$
	Xy	$2+p$	2	5	5	$9-(1-p)(1-q)$
	xY	p	0	1	1	$1-(1-p)(1-q)$
	xy	$1-p$	1	1	1	$(1-p)(1-q)$
4. $(\mathbf{Xy})_2.\mathbf{xY}.\mathbf{xy}$	XY	$2+p$	2	5	5	$26+pq$
	Xy	$3-p$	3	5	5	$9-pq$
	xY	$1-p$	1	1	1	$1-pq$
	xy	p	0	1	1	pq
5. $(\mathbf{XY})_2.(\mathbf{xy})_2$	XY	$5-2p+p^2$	5	17	25	$34+(1-p)^2(1-q)^2$
	Xy	$2p-p^2$	0	3	5	$1-(1-p)^2(1-q)^2$
	xY	$2p-p^2$	0	3	5	$1-(1-p)^2(1-q)^2$
	xy	$1-2p+p^2$	1	1	1	$(1-p)^2(1-q)^2$
6. $(\mathbf{Xy})_2.(\mathbf{xY})_2$	XY	$4+p^2$	4	17	25	$34+p^2q^2$
	Xy	$1-p^2$	1	3	5	$1-p^2q^2$
	xY	$1-p^2$	1	3	5	$1-p^2q^2$
	xy	p^2	0	1	1	p^2q^2
7. $\mathbf{XY}.\mathbf{Xy}.\mathbf{xY}.\mathbf{xy}$	XY	$8+p-p^2$	4	33	25	$136+pq(1-p)(1-q)$
	Xy	$2-p+p^2$	1	7	5	$4-pq(1-p)(1-q)$
	xY	$2-p+p^2$	1	7	5	$4-pq(1-p)(1-q)$
	xy	$p-p^2$	0	1	1	$pq(1-p)(1-q)$

For purposes of calculation it is convenient to put $1-p=P$, $1-q=Q$.
The gametic series in double coupling may then be written $4+P^2 : 1-P^2 : 1-P^2 : P^2$, and the expressions for the following zygotic series may be simplified:

Single coupling, $2+PQ : 1-PQ : 1-PQ : PQ$.
Asymmetrical coupling, $26+PQ : 9-PQ : 1-PQ : PQ$.
Double coupling, $34+P^2Q^2 : 1-P^2Q^2 : 1-P^2Q^2 : P^2Q^2$.
Coupling and repulsion, $136+pqPQ : 4-pqPQ : 4-pqPQ : pqPQ$.

The second column gives the gametic series in general, the third the expected ratios when linkage is so strong that crossing-over may be neglected, the fourth when linkage is so weak that crossing-over amounts to 50 per cent. In the fifth column the ratios are given which are found when the factors are in different chromosomes. In the last column are given the zygotic ratios to be expected on selfing. In the case of single coupling the ratios are the same as in a diploid. But in the case of single repulsion this is not so, since two factors in different chromosomes can still enter the same gamete. The asymmetrical cases call for no special comment.

Whereas in the first four cases no difference is to be expected when the factors, though in the same chromosome, are far apart, from the ratios obtained when they are in different chromosomes, this is not so in the last three. If the factors are in different chromosomes each tetrad of homologous chromosomes can pair in three ways giving two pairs each, so the total number of distinct cases to be considered is 36. But if the factors are far apart in the same chromosome each of the six possible pairs of chromosomes can produce one, two or four different types of gametes; the total number of cases is therefore 24 or 48. It is thus theoretically possible, in a tetraploid plant, to distinguish between 50 per cent. crossing-over and the absence of linkage.

Similar calculations have been made to meet the possibility that, after pairing, the chromosomes can enter gametes at random, so that in one-third of all cases, two chromosomes which have paired so as to permit of crossing-over may enter the same gamete. The expected ratios are somewhat different. As, however, it will be shown that this event occurs rarely if ever in *Primula sinensis*, the possibility need not be further considered here. It is however possible that it may occur in other tetraploid organisms, or that a state of affairs may be found in them intermediate between the above condition and that here described. If after pairing, chromosomes always went to the same pole, only **XY** and **xy** gametes would be found in the case of single or double coupling. This, of course, is not the case.

When three factors are concerned, matters are much more complicated: 44 possible zygotic types must be considered. Moreover two different types of double crossing-over are theoretically possible. Consider four homologous chromosomes *A, B, C, D*, in a zygote

$$\begin{aligned} &\textbf{XYZ}\\ &\textbf{xyz}\\ &\textbf{xyz}\\ &\textbf{xyz} \end{aligned}$$

Chromosome A may pair with B, crossing-over twice, and giving **XyZ** and **xYz** gametes. Or it may pair with both B and C, giving **Xyz**, **xYz** and **xyZ**. Both these types of crossing-over were found by Bridges and Anderson (1925) in the triploid *Drosophila melanogaster*, the first being termed recurrent, the second progressive. In the case of progressive crossing-over in a tetraploid we should expect that, as the result of a situation such as that shown in Fig. 1, the two gametes formed would be
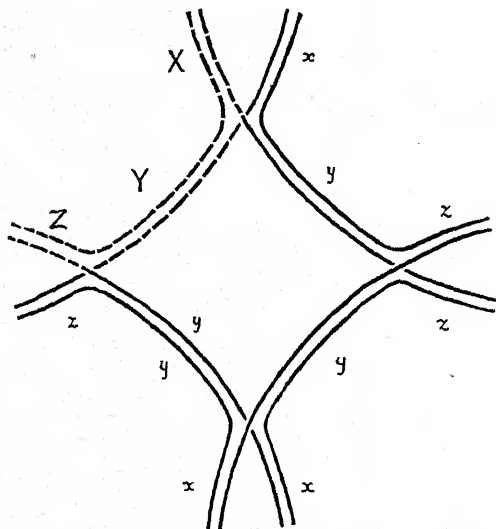
Fig. 1. Configuration in the diplotene stage which might yield **Xyz** gametes.
                                                            xyZ
The chromosome containing the three dominants is dotted.

xYz and **Xyz**. The fact that in such a case **X** and **Z** would exhibit re-
xyz   **xyZ**
pulsion in the progeny shows that such cases, if they occur, are rare. If they are at all frequent, however, **XyZ** and **xYz** gametes should be more commonly produced by the zygote

$$
\begin{array}{c}
\textbf{XYZ} \\
\textbf{xyz} \\
\textbf{xyz} \\
\textbf{xyz}
\end{array}
$$

than by **XYZ**, provided that the cross-over values are the same in both.
        xyz
Double cross-overs are not more common in the tetraploid, not at least to any significant extent. Hence it is considered that, for the present, a theory

based on crossing-over involving two chromosomes only, which is not presented as final, will cover all the facts found, to a first approximation.

In view of the somewhat complicated nature of the theory, it is perhaps worth pointing out simpler, if less rigorous, methods of calculating some of the above gametic and zygotic series. Consider the case of double repulsion. The **yy** gametes form one-sixth of the whole, from the single factor theory. But in order that a **yy** gamete should also be **xx**, crossing-over must have occurred twice. The chance of this is p^2: hence the ratio of **Xyy** to **xxyy** gametes is $1 - p^2 : p^2$. The relative proportion of **xxY** gametes is also $1 - p^2$. But since the total proportion of **X** gametes is five for every one **xx**, there must be $4 + p^2$ **XY** gametes. Similarly the proportion of **xxyy** gametes in double coupling is P^2, this being the chance that no crossing-over has occurred on two distinct occasions.

In the corresponding zygotic series the proportions of bottom recessives are $\dfrac{p^2q^2}{36}$ and $\dfrac{P^2Q^2}{36}$. In the first case crossing-over must have occurred twice on the male and twice on the female side (a most improbable occurrence), in the second it must have failed to occur on four independent occasions. So that even in a doubly coupled zygote, double recessives rarely occur as a result of selfing. Thus if the cross-over value in each sex is $33\frac{1}{3}$ per cent., $p = \frac{1}{3}$, $P = \frac{2}{3}$, therefore the proportion of double recessives is $\dfrac{1}{36}\left(\dfrac{2}{3}\right)^4$, or 4 in 729. This is greater than the proportion of 1 in 1296 expected in the absence of linkage, but is still small.

EXPERIMENTAL RESULTS.

In the interpretation of the experimental results there is a certain danger of circularity in the argument. Thus a number of plants, from their genetical behaviour, agree with the expectation on the assumption that they are of the composition

SBG
sbg
sbg
sbg

Some of these plants are derived from a cross between a triple recessive plant and a triple dominant of uncertain composition. Others arise from selfing, and so on. Only their genetical behaviour makes their composition more or less certain. On the other hand, many such plants are derived from the cross of a plant known from its ancestry, genetical behaviour, or both, to be of the above composition, crossed with a triple

recessive. On the theory here developed, all plants in such families, provided that they carry the three dominants, should be of the above composition, unless some exceptional event such as progressive crossing-over has occurred. Such plants, and no others, may legitimately be used to test the theory.

We may however legitimately include with them other plants of similar genetical behaviour, provided their offspring occur in the theoretical proportions, and use the total of such families for the purpose of calculating linkage intensities. In the tables three distinct classes of parents are considered.

(*a*) Parents whose genetic composition can be predicted from those of their ancestors on the assumption that neither double reduction, non-disjunction, nor progressive crossing-over has occurred.

(*b*) Parents in which the ancestry does not suffice to determine the number of factors, but where the linkages are certain once the numbers of factors are known. Now the number of factors (*i.e.* whether the plant is simplex or duplex) can be determined from the ratios in which the single factors segregate in their progeny. In families of ten or more plants there is very little chance of confusing a $1:1$ with a $5:1$ ratio, and little chance of confusing a $3:1$ with a $35:1$. Hence the progeny of such plants furnish reliable data on linkage. For example $161^1/24$ was derived from a cross between **SSSsBBbb** and **ssssbbbb**, and being a thrum magenta, was either duplex or simplex in **S** and **B**. Crossed with an **ssssbbbb** plant it gave 18**S**, 2**s** and 7**B**, 13**b**. Hence its composition was **SSssBbbb**. But since one of the gametes which formed it was **ssbb**, its own composition was necessarily

$$\begin{array}{c} \mathbf{SB} \\ \mathbf{Sb} \\ \mathbf{sb} \\ \mathbf{sb} \end{array}$$

Most of our data on asymmetrical linkage are derived from such plants, though it would be theoretically possible to make them from a cross between **SSSSSBbbb** (derived from a cross of **SSSSSBbbb** and **SSSSbbbb**) and **ssssbbbb**. Such a parent would be of class (*a*).

(*c*) Parents whose composition is only deducible from their offspring. As an example

$$\begin{array}{c} \mathbf{SBG} \\ \mathbf{sbg} \\ \mathbf{sbg} \\ \mathbf{sbg} \end{array}$$

selfed has given several plants which behaved as

SBG
SBG
sbg
sbg

But even after the single-factor ratios were determined they might as well have been of composition

SBG or of SBg
SBg   SBg
sbG   sbG
sbg   sbG

to name only two possibilities.

We are quite aware that the inclusion of class (b) leads to a certain distortion of the single factor ratios, and class (c) may also distort the linkage ratios, owing to the omission, in each case, of parents of uncertain composition. Nevertheless we think their inclusion justifiable, with the above caution. It will be seen that even if attention is confined to the class (a) plants, the general features of the linkage are quite clear. We have allowed ourselves the inclusion of a few families of 1930 which are not quite complete, certain plants not yet having flowered. These are not included in the totals of Table II, but we consider that on the whole more is gained than lost by including them in Tables IV–XI.

Origin of the plants considered.

Tetraploids of the following origins have been used:

(a) Gregory's (1914) **GX** race which originated in a plant from Messrs Sutton in 1909. This plant lacked **S** and was at least duplex for **B, G, D** (white), and **Y** (palmate as opposed to fern leaf).

(b) Gregory's (1914) **GT** race which originated in 1911 from a cross made by him between two diploids. It was at least duplex for **S, B** and **G**, and also carried **D**.

(c) Sutton's "Symmetry," introduced into the experiments in 1920, and Sutton's "Mosscurl" in 1922. Both lacked **S** and **B** and were at least triplex for **G**. Most of our numbers for **S, G** asymmetrical repulsion come from these races crossed to others lacking **G** and simplex for **S**.

Linkage between two factors.

The single coupling figures (Table IV) require little comment. Only two of the families considered are in any way abnormal. Among the class (a) families from **SB** . $(sb)_3 \times (sb)_4$ occurs one (149/22) consisting of

19 SB, 20 Sb, 11 sB, 19 sb. It seems probable that its parent 43¹/21 was of the composition mentioned, and it behaved more normally in another similar cross, giving 5 SB, 1 Sb, 1 sB, 2 sb. It is clear, however, that

TABLE IV.

$$\text{Single coupling } \frac{XY}{xy} \frac{}{} \frac{xy}{xy}$$

Parents*		No. of families	SB	Sb	sB	sb
SB.(sb)₃ × (sb)₄	(a)	35	475	59	45	453
,, ,,	(b)	19	191	21	12	148
,, ,,	(c)	2	101	6	11	83
,, ,,	(Total)	56	767	86	68	684
,, ,,	(Calc.)	—	738·2	64·3	64·3	738·2
(sb)₄ × SB.(sb)₃	(a)	24	229	25	23	303
,, ,,	(b)	15	88	9	8	72
,, ,,	(c)	2	13	1	0	14
,, ,,	(Total)	41	330	35	31	389
,, ,,	(Calc.)	—	359·5	33	33	359·5
SB.(sb)₃ × SB.(sb)₃	(a)	14	304	18	17	96
,, ,,	(b)	8	145	7	8	53
,, ,,	(c)	7	137	4	9	40
,, ,,	(Total)	29	586	29	34	189
,, ,,	(Calc.)	—	595·5	33·0	33·0	176·5
			SG	Sg	sG	sg
SG.(sg)₃ × (sg)₄	(a)	15	178	114	101	169
,, ,,	(b)	2	26	12	12	24
,, ,,	(Total)	17	204	126	113	193
,, ,,	(Calc.)	—	198·5	119·5	119·5	198·5
(sg)₄ × SG.(sg)₃	(a)	16	124	80	95	155
,, ,,	(b)	4	8	9	2	5
,, ,,	(Total)	20	132	89	97	160
,, ,,	(Calc.)	—	146	93	93	146
SG.(sg)₃ × SG.(sg)₃	(a)	7	179	47	40	27
,, ,,	(Calc.)	—	174·4	45·3	45·3	27·9
			BG	Bg	bG	bg
BG.(bg)₃ × (bg)₄	(a)	16	190	105	93	180
,, ,,	(b)	4	33	16	21	30
,, ,,	(Total)	20	223	121	114	210
,, ,,	(Calc.)	—	216·5	117·5	117·5	216·5
(bg)₄ × BG.(bg)₃	(a)	16	135	69	84	165
,, ,,	(b)	4	8	9	2	5
,, ,,	(Total)	20	143	78	86	170
,, ,,	(Calc.)	—	156·5	82	82	156·5
BG.(bg)₃ × BG.(bg)₃	(a)	7	177	48	42	26
,, ,,	(b)	2	14	1	4	2
,, ,,	(Total)	9	191	49	46	28
,, ,,	(Calc.)	—	190·4	45·1	45·1	33·4

* Throughout these tables the parent used as a female is put first. The groups in which the parents are of the same composition almost all arise from self-fertilisation.

some anomaly is occurring here (possibly a mistake was made in crossing). The chance of obtaining such a family by random sampling is much less than one in a billion. It has therefore been omitted from the total figures used in calculating the **SB** cross-over value of 8·01 per cent. Its in-

TABLE V.

Single repulsion Xy
xY
xy
xy

Parents		No. of families	SB	Sb	sB	sb
Sb.sB.(sb)₂×(sb)₄	(a)	3	36	43	39	28
" "	(c)	1	8	12	6	6
" "	(Total)	4	44	55	45	34
" "	(Calc.)	—	32	57	57	32
(sb)₄×Sb.sB.(sb)₂	(a)	3	11	25	15	15
" "	(Calc.)	—	11·9	21·1	21·1	11·9
Sb.sB.(sb)₂×Sb.sB.(sb)₂	(b)	2	40	17	11	0
" "	(Calc.)	—	36·2	14·8	14·8	2·2
			SG	**Sg**	**sG**	**sg**
Sg.sG.(sg)₂×(sg)₄	(a)	11	72	76	77	61
" "	(b)	12	54	60	69	41
" "	(Total)	23	126	136	146	102
" "	(Calc.)	—	116·9	138·1	138·1	116·9
(sg)₄×Sg.sG.(sg)₂	(a)	9	31	43	46	40
" "	(b)	5	7	14	14	12
" "	(Total)	14	38	57	60	52
" "	(Calc.)	—	47·9	55·6	55·6	47·9
			BG	**Bg**	**bG**	**bg**
Bg.bG.(bg)₂×(bg)₄	(a)	7	32	32	38	31
" "	(b)	10	47	48	62	37
" "	(Total)	17	79	80	100	68
" "	(Calc.)	—	73·7	89·8	89·8	73·7
(bg)₄×Bg.bG.(bg)₂	(a)	4	10	22	30	24
" "	(b)	5	6	14	15	12
" "	(Total)	9	16	36	45	36
" "	(Calc.)	—	29·8	36·7	36·7	29·8
Bg.bG.(bg)₂×Bg.bG.(bg)₂	(c)	1	8	5	4	1
" "	(Calc.)	—	9·9	3·6	3·6	0·9

clusion would bring this value up to 9·53 per cent. Another plant 180³/28, believed from its ancestry to be **SBG**.(sbg)₃, when used as a female with (sbg)₄ gave normal coupling of **S** and **B**, but 7 **SG**, 10 **Sg**, 9 **sG**, 6 **sg** and 6 **BG**, 10 **Bg**, 10 **bG**, 6 **bg**, as if **G** were being repelled from **S** and **B**. As male and when selfed the numbers were too small to be decisive. However, three **SBG** plants from the anomalous family 70–73/29 whose numbers are given

above were selfed, and all behaved as if **SBG** were coupled, the totals being 29 **SG**, 10 **Sg**, 17 s**G**, 7 sg. The family 70–73:29 is therefore included, although the proportion of cross-overs between **B** and **G** deviates by more than six times the standard error of sampling. It must be realised, however, that cross-over values probably do vary in reality, and not only as a result of sampling.

The calculated figures are obtained directly from the back-cross data, so that the agreement in the case of back-crosses is no proof of the correctness of the theory. The agreement is, however, quite satisfactory in the case of the families due to selfing. The inclusion of class (*c*) plants only alters the **SB** cross-over values from 7·93 to 8·01, and from 8·59 to 8·41 per cent.

The data for single repulsion are given in Table V. The figures are decidedly irregular, but this is mainly due to the single factor ratios. If there is any systematic difference between observed and calculated linkage, it should show up in a difference between observed and calculated numbers of **XY** + **xy** zygotes (*i.e.* **SB** + **sb**, etc.) in the crosses giving 1 : 1 single factor ratios. The observed numbers are 621 (**XY** + **xy**), 800 (**Xy** + **xY**), the calculated 624·4 and 796·6. The agreement is thus very good, and the theory as a whole is confirmed. It is at once clear that the phenomenon is quite different from repulsion in a diploid.

In the case of asymmetrical coupling and repulsion a number of families have not been included which illustrate linkage of **G** with **S** and **B**. These are class (*c*) families. That is to say, the type of linkage is deduced from the family concerned, and is not certain from the ancestry. In the case of linkage between **S** and **B**, however, such families are included. The linkage being strong, there is little chance of mistaking coupling for repulsion, especially when the plant whose composition is doubtful has been both crossed and selfed. Except in the case of the asymmetrical repulsion of **S** and **G**, which arose from frequent crosses between horticultural varieties homozygous for **S** and **G**, and Ss_3g_4 plants, the data are scrappy, and it is difficult to be sure how far the disagreements of theory and observation are fortuitous.

Summing the asymmetrical coupling figures from

$$XY \cdot Xy \cdot (xy)_2 \times (xy)_4$$

and the reciprocal we have:

	XY	Xy	xY	xy
Found	598	431	33	138
Calculated	544·5	455·9	55·9	144·1

TABLE VI.

Asymmetrical coupling **XY**
Xy
xy
xy

Parents		No. of families	SB	Sb	sB	sb
SB.Sb.(sb)₂ × (sb)₄	(b)	1	6	12	1	1
,, ,,	(c)	7	49	32	0	11
,, ,,	(Total)	8	55	44	1	12
,, ,,	(Calc.)	—	54·5	38·8	1·5	17·2
SB.sB.(sb)₂ × (sb)₄	(b)	8	100	1	51	18
,, ,,	(c)	1	18	0	13	11
,, ,,	(Total)	9	118	1	64	29
,, ,,	(Calc.)	—	103·2	2·8	73·5	32·5
(sb)₄ × SB.Sb.(sb)₂	(c)	6	22	11	0	6
,, ,,	(Calc.)	—	18·9	13·6	0·55	5·9
(sb)₄ × SB.sB.(sb)₂	(c)	1	2	0	2	0
,, ,,	(Calc.)	—	2·1	0·05	1·4	0·6
SB.Sb.(sb)₂ × SB.Sb.(sb)₂	(b)	3	33	14	1	1
,, ,,	(c)	1	2	1	0	0
,, ,,	(Total)	4	35	15	1	1
,, ,,	(Calc.)	—	38·8	11·8	0·2	1·2
SB.sB.(sb)₂ × SB.sB.(sb)₂	(b)	5	71	1	32	0
,,	(Calc.)	—	77·5	0·5	23·6	2·4

Parents		No. of families	SG	Sg	sG	sg
SG.Sg.(sg)₂ × (sg)₄	(b)	3	26	15	3	5
,, ,,	(Calc.)	—	21·4	19·4	3·1	5·1
SG.sG.(sg)₂ × (sg)₄	(a)	1	3	0	4	2
,, ,,	(b)	13	153	9	104	33
,, ,,	(Total)	14	156	9	108	35
,, ,,	(Calc.)	—	134·7	19·3	122	32
(sg)₄ × SG.sG.(sg)₂	(a)	1	12	5	20	7
,, ,,	(b)	4	36	2	30	6
,, ,,	(Total)	5	48	7	50	13
,, ,,	(Calc.)	—	51·4	7·7	47	12
SG.sG.(sg)₂ × SG.sG.(sg)₂	(a)	3	101	1	30	2
,, ,,	(b)	7	272	6	88	8
,, ,,	(Total)	10	373	7	118	10
,, ,,	(Calc.)	—	371·2	9·8	121·6	5·4

Parents		No. of families	BG	Bg	bG	bg
BG.bG.(bg)₂ × (bg)₄	(b)	10	122	7	88	23
,, ,,	(Calc.)	—	105·9	14·1	94·1	25·9
(bg)₄ × BG.bG.(bg)₂	(a)	1	12	3	20	9
,, ,,	(b)	4	37	2	29	6
,, ,,	(Total)	5	49	5	49	15
,, ,,	(Calc.)	—	52·2	6·8	46·1	12·9
BG.bG.(bg)₂ × BG.bG.(bg)₂	(b)	8	175	5	70	4
,,	(Calc.)	—	186·4	4·1	60·5	3

The disagreement in the case of the Small cross over class **xY** is serious. But it is partly due to the very bad single factor ratio for **X**, the number of recessives being only 171 instead of 200. Making allowance for this the number of **xY** would be increased to 39·3. The divergence is now only just over twice the standard error, and not certainly significant. Only further work can decide whether the theory holds in this case. On the other hand in the case of selfed plants we have:

	XY	Xy	xY	xy
Found	654	235	14	15
Calculated	675·9	218·6	13·5	12·0

The agreement is much better, and the cross-overs are in excess of expectation, which suggests that the disagreement in the former case is due to bad luck.

In the case of asymmetrical repulsion, theory agrees very well with observation. The figures could be used to calculate linkage intensity. Thus if, the expectation being $3 - p : 2 + p : 1 - p : p$, the numbers found are a, b, c and d, the method of maximum likelihood (cf. Fisher and Balmukand (1928)) shows that p is a root of

$$\frac{a}{p-3} + \frac{b}{p+2} + \frac{c}{p-1} + \frac{d}{p} = 0,$$

or $$(a + b + c + d) p^3 - (-a + 4b + c + 2d) p^2$$
$$- (2a - 3b + 6c + 5d) p + 6d = 0.$$

Applying this equation to the data of $Sg.(sG)_2.sg.(sg)_4$, where $a = 439, b = 422, c = 98, d = 70$, we have:

$$1029p^3 - 1487p^2 - 550p + 420 = 0,$$

whence $p = 42\cdot2$ per cent. as compared with 37·6 per cent. from the single coupling data. We have not however used such figures to correct the linkage values used, since the agreement of observation and calculation is more logically demonstrated when the latter is based on single coupling only.

The majority of figures for double coupling of **S** and **B** come from a few plants which are placed in class (c) but whose composition is not really in the least doubt. They were derived from the self-fertilisation of known $SB.(sb)_3$ plants, and have given large families which make it clear that they are of the composition $(SB)_2.(sb)_2$. Actually of the $S_2s_2B_2b_2$ plants from such ancestry 84 per cent. should be of the above composition, and the remainder should give many more **Sb** and **sB** than **sb** plants when crossed to a recessive. The progeny of two of these plants is also included in the tables for double coupling of **G** with **S** and **B**, but the

TABLE VII.

Asymmetrical repulsion Xy
Xy
xY
xy

Parents		No. of families	SG	Sg	sG	sg
Sg.(sG)$_2$.sg × (sg)$_4$	(a)	25	260	64	297	45
,, ,,	(b)	19	162	34	142	25
,, ,,	(Total)	44	422	98	439	70
,, ,,	(Calc.)	—	407·5	107	450	64·5
(sg)$_4$ × Sg.(sG)$_2$.sg	(a)	8	24	6	31	4
,, ,,	(b)	2	9	1	7	0
,, ,,	(Total)	10	33	7	38	4
,, ,,	(Calc.)	—	32·7	8·3	35·7	5·3
Sg.(sG)$_2$.sg × Sg.(sG)$_2$.sg	(a)	12	520	25	152	5
,, ,,	(b)	13	327	6	115	2
,, ,,	(Total)	25	847	31	267	7
,, ,,	(Calc.)	—	836·8	27·2	283·2	4·8
			BG	Bg	bG	bg
Bg.(bG)$_2$.bg × (bg)$_4$	(b)	3	39	8	39	10
,, ,,	(Calc.)	—	37·5	10·4	42·5	5·6
(bg)$_4$ × Bg.(bG)$_2$.bg	(b)	4	13	2	8	0
,, ,,	(Calc.)	—	9	2·5	10·2	1·3
(Bg)$_2$.bG.bg × (Bg)$_2$.bG.bg	(b)	2	69	32	3	1
,, ,,	(Calc.)	—	76·6	25·9	2·1	0·35
Bg.(bG)$_2$.bg × Bg.(bG)$_2$.bg	(a)	1	15	3	9	1
,, ,,	(b)	3	24	1	9	0
,, ,,	(Total)	4	39	4	18	1
,, ,,	(Calc.)	—	45	1·5	15·3	0·21

propriety of this step is less certain. The total number of cross-overs, 118, is less than the expectation, 134·2, but not sufficiently so to warrant the deduction that crossing-over between one pair of chromosomes hinders simultaneous crossing-over between the other pair. Certainly, however, there is no suggestion of a positive correlation between the two processes.

The double repulsion figures are less satisfactory. Nevertheless the class (b) families demonstrate the existence of the phenomenon in the case of **B** and **G**. Some of the class (c) families here included may really be examples of the seventh type of linkage, viz. coupling with repulsion. However, in each case considerations favour the assignment here given. There is possibly, as in the last case, a deficiency of the zygotic type (here the double recessive) which is due to simultaneous crossing-over. Besides the families of Tables VIII and IX a large number of other families are on record which are derived from parents duplex for two factors. But the evidence regarding their linkage is quite inconclusive. They may in most cases be examples either of double coupling, of double repulsion or

of coupling and repulsion. Unfortunately we have as yet no clear case of the latter type of linkage.

We are aware that the data on double coupling and repulsion are unsatisfactory. In order to remedy this defect it is proposed to establish pure (*i.e.* quadruplex) lines of dominants. Since, however, two generations are required to test the homozygosity of such lines, satisfactory data will not be available for some years, and it has been thought best to publish the present evidence, which clearly demonstrates the existence of double coupling and repulsion, although the precise laws which they obey are still in some doubt.

TABLE VIII.

Double coupling $\dfrac{XY}{XY}$ $\dfrac{xy}{xy}$

Parents		No. of families	SB	Sb	sB	sb
$(SB)_2.(sb)_2 \times (sb)_4$	(b)	1	14	1	0	0
,, ,,	(c)	7	274	7	4	54
,, ,,	(Total)	8	288	8	4	54
,, ,,	(Calc.)	—	285·9	9·1	9·1	49·9
$(sb)_4 \times (SB)_2.(sb)_2$	(c)	2	84	5	3	16
,, ,,	(Calc.)	—	87·1	2·9	2·9	15·1
$(SB)_2.(sb)_2 \times (SB)_2.(sb)_2$	(c)	5	146	1	0	3
,, ,,	(Calc.)	—	144·6	1·2	1·2	3·0
			SG	Sg	sG	sg
$(SG)_2.(sg)_2 \times (sg)_4$	(b)	3	16	0	3	1
,, ,,	(c)	3	130	18	10	17
,, ,,	(Total)	6	146	18	13	18
,, ,,	(Calc.)	—	142·7	19·8	19·8	12·7
$(sg)_4 \times (SG)_2.(sg)_2$	(b)	1	45	6	3	11
,, ,,	(c)	1	36	7	6	6
,, ,,	(Total)	2	81	13	9	17
,, ,,	(Calc.)	—	87·5	12·5	12·5	7·5
$(SG)_2.(sg)_2 \times (SG)_2.(sg)_2$	(b)	3	80	2	2	2
,, ,,	(c)	1	24	1	2	0
,, ,,	(Total)	4	104	3	4	2
,, ,,	(Calc.)	—	107·2	2·7	2·7	0·45
			BG	Bg	bG	bg
$(BG)_2.(bg)_2 \times (bg)_4$	(c)	1	101	13	9	13
,, ,,	(Calc.)	—	100·2	13·1	13·1	9·5
$(bg)_4 \times (BG)_2.(bg)_2$	(c)	1	36	7	6	6
,, ,,	(Calc.)	—	40·6	5·2	5·2	3·9
$(BG)_2.(bg)_2 \times (BG)_2.(bg)_2$	(c)	1	24	0	2	1
,, ,,	(Calc.)	—	25·6	0·61	0·61	0·14

TABLE IX.

Double repulsion **Xy**
Xy
xY
xY

Parents		No. of families	SG	Sg	sG	sg
$(Sg)_2 . (sG)_2 \times (sg)_4$	(c)	12	124	21	19	0
,, ,,	(Calc.)	—	113·3	23·4	23·4	3·9
$(sg)_4 \times (Sg)_2 . (sG)_2$	(c)	2	11	0	1	0
,, ,,	(Calc.)	—	8·3	1·7	1·7	0·30
			BG	**Bg**	**bG**	**bg**
$(Bg)_2 . (bG)_2 \times (bg)_4$	(b)	5	110	24	14	2
,, ,,	(c)	3	40	14	8	0
,, ,,	(Total)	8	150	38	22	2
,, ,,	(Calc.)	—	145·7	31·0	31·0	4·4
$(bg)_4 \times (Bg)_2 . (bG)_2$	(b)	1	4	0	0	0
,, ,,	(Calc.)	—	2·7	0·54	0·54	0·08
$(Bg)_2 . (bG)_2 \times (Bg)_2 . (bG)_2$	(b)	5	102	1	1	0
,, ,,	(Calc.)	—	98·2	2·8	2·8	0·04

Linkages between three factors.

The totals of 30 families, all of class (*a*), in which all three factors were singly coupled, are collected in Table X. In 15 the cross was

$$\mathbf{SBG} . (\mathbf{sbg})_3 \times (\mathbf{sbg})_4,$$

in the other 15 $(\mathbf{sbg})_4 \times \mathbf{SBG} . (\mathbf{sbg})_3.$

The ratios expected are the same as in diploid linkage. The expectations given in the table are calculated from the linkage values found for the factors two at a time. The agreement found merely shows that the families considered are a fair sample.

The interest centres on the double cross-overs, which are fewer than expected. If there were no interference, *i.e.* if crossing-over between **S** and **B** did not diminish the probability of crossing-over between **B** and **G**, the expected values in these families would be $\frac{41 \times 183}{543}$, or 13·8 on the female side, and $\frac{29 \times 147}{420}$, or 10·15 on the male side. The average coincidence is thus 67 per cent., most marked on the male side. On the basis of the cross-over values of Table I the coincidence is 99 per cent. on the female side, and 69 per cent. on the male side. But these estimates are less reliable because the figures used are not all drawn from the same families. In the diploid the data of Gregory, de Winton and Bateson give a coincidence of 89 per cent. on the female side and 83 per cent. on the male side. The concordance is quite satisfactory in view of the small

numbers. There is no suggestion that double crossing-over is easier in the tetraploid owing to exchanges involving three chromosomes.

TABLE X.

Progeny of **SBG** . **(sbg)**$_3$ × **(sbg)**$_4$ *and reciprocally.*

		Ex Het. ♀	Ex Het. ♂
SBG sbg	———	173} 157} 330 (323·7)	112} 137} 249 (248·6)
Sbg sBG	———	15} 15} 30 (28·3)	11} 13} 24 (27·0)
SBg sbG	———	92} 80} 172 (175·8)	66} 76} 142 (136·0)
SbG sBg	———	5} 6} 11 (15·2)	2} 3} 5 (8·4)

TABLE XI.

SBG . **(sbg)**$_3$ *selfed. Six families.*

	SBG	SBg	SbG	Sbg	sBG	sBg	sbG	sbg
Found	169	45	10	2	8	3	32	24
Calculated	169·2	39	5·2	6·3	8·4	3·1	36·9	24·8

TABLE XII.

Sbg . **sBG** . **(sbg)**$_2$ × **(sbg)**$_4$ *and reciprocally.*

SBG sbg	14} 11}	$25 (23·5 = 1 + x - y - z)$
SBg . sbG	10} 10}	$20 (12·2 = y + 2z)$
SbG sBg	10} 14}	$24 (22·1 = 2y + z)$
Sbg sBG	18} 12}	$30 (41·2 = 2 - x - 2y - 2z)$

In a diploid, satisfactory data regarding the linkage of three factors can be obtained even when one is repelled from the other two. This is not so in a tetraploid. Four class (*a*) families are derived from the mating **Sbg** . **sBG** . **(sbg)**$_2$ × **(sbg)**$_4$, and from the reciprocal cross. They are summarised in Table XII. The expectation is calculated on the basis that x is the expected proportion of cross-overs between the loci of **S** and **B**, y between those of **B** and **G**, and z the proportion of double cross-overs. The values taken, weighted to allow for the fact that reciprocal crosses are added together, are $x = 0·0582$, $y = 0·3220$, $z = 0·0243$. It will be seen that the agreement of theory and expectation is poor. But it is also clear that such data would be useless for calculating z. Other families exist in which **S**, **B** and **G** were all in different chromosomes. But they are mostly in class (*b*), and cannot be expected to agree very well with theory; nor do they throw any light on double crossing-over.

In Table XI are collected six families, all of class (a), from the selfing of **SBG**.(**sbg**)$_3$ plants. The agreement with theory is on the whole good. The only class which is necessarily due to double crossing-over of one chromosome is **sBg**. **SbG** can of course be formed by one cross-over on the female side and another on the male side.

Discussion.

It is at once clear that the various results obtained agree fairly well with expectation on the chromosome theory. In fact the agreement is rather surprising in view of the numerous irregularities in meiosis which Darlington (1930) has described in this plant. We should have expected to find single factor ratios nearer to those deduced from a basis of random segregation between chromatids, and also evidence of crossing-over involving three chromosomes, double reduction, and other anomalies. The regularity observed may be due to several causes. The chromosome considered may have little tendency to form quadrivalents at meiosis. This is rather unlikely, as it contains 6 of the 27 known factors, and is therefore probably fairly long.

A somewhat more likely view, suggested to me by Dr Darlington, is that the three factors in question are located rather near the attachment constriction of the chromosome in which they are situated. In this case segregation would be little affected by the fact that pairing is between chromatids, and not whole chromosomes. The only common type of non-disjunction to be expected in such a case would be that leading to $2n + 1$ or $2n - 1$ gametes, which are doubtless largely eliminated, though as pointed out earlier in the paper, it is probable that some of our parent plants possessed an extra chromosome in the set carrying the factors discussed.

Table I gives a comparison of linkage values. It is obvious that the difference in linkage intensity which exists in the diploid between the male and the female sides of the plant is here absent or very slight. In each case the tetraploid values found are intermediate between those found on the two sides of the diploid. The differences, however, are not always large compared with their standard errors; but that between the cross-over values for **S** and **B** on the male side is 4·50 per cent., with a standard error of only 1·11 per cent.; the difference being four times its standard error, the odds in favour of its significance are nearly 10,000 to 1, assuming the errors to be due to sampling only. Even though, as pointed out above, a few families are aberrant, so that errors are not solely due to sampling, the difference is probably real.

The work is being continued. Recent observations, both on the

diploid and tetraploid, have made it probable that the factor **V**, in whose absence the stem is green, is linked with **S**, **B** and **G**. We hope to compare this linkage in the diploid and tetraploid. The linked factors **F** (for flat as opposed to crimped leaves) and **Ch** (for *sinensis* as opposed to *stellata* type) are also available in the tetraploid. But the dominance of both is so incomplete as to render them unsuitable for accurate work. Before the theory here given can be regarded as generally applicable to autotetraploids it is desirable that it should be tested on other plants. Dr Sansome is at present engaged in a study of linkage in the tetraploid tomato at this Institution.

SUMMARY.

1. An account is given of six types of linkage observed between three pairs of factors in the tetraploid *Primula sinensis*, and of a seventh theoretically possible type.

2. The intensity of linkage is nearly, but not quite, the same in the tetraploid as in the diploid. It is the same on the two sides in the tetraploid.

3. As regards the factors considered, there is no evidence of crossing-over involving more than two chromosomes at a time, or of two chromosomes going to the same pole after crossing-over.

4. The six readily available gametic series contain only one adjustable constant p, and since the experimental results in other cases agree reasonably well with prediction when p has been calculated from the results of single coupling, this affords substantial support of the chromosome theory of inheritance.

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MEIOSIS IN A TRIPLOID *OENOTHERA*.

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(With Twelve Text-figures.)

IT was reported (Catcheside, 1930) that diploid *Oenothera pycnocarpa* had a circle of fourteen chromosomes, and that a triploid plant, in the same culture, and apparently not markedly different from the rest, had a circle of twenty-one chromosomes, a circumstance at variance with the requirements of Darlington's (1929 *a*) hypothesis of segmental interchange. A formula using the latter's conventions was submitted, duplication of a haploid group, representing one of the complexes, being assumed.

After seeing my preparations, Dr Darlington was of the opinion that a closed ring was not present, and states, in a letter to *Nature* (1930 *a*), that he could make out the following associations: (i) unpaired chromosomes; (ii) rod pairs, united at one end; (iii) ring pairs, united at both ends; (iv) chains of three and of four chromosomes; (v) branched chains of chromosomes (with triple union); (vi) ring pairs associated (by a triple union) with one end of a third chromosome. He remarks further, rightly, that these types of configuration are in agreement with the formula given.

Observation in this triploid is difficult at diakinesis and the heterotypic metaphase and, in view of the divergent opinion of Darlington, another examination of the material was undertaken; the present illustrations were made at table level, with the aid of a camera lucida, using a 2 mm. N.A. 1·3 Zeiss homog. imm. lens combined with a × 20 comp. ocular, giving a magnification of × 3300.

The results detailed below are different from those obtained previously; they amply confirm Darlington's (1930 *a*) statements, and must lead to different conclusions. The previous misstatements must be traced to faulty interpretation, coupled perhaps with a prejudiced preconception.

Earlier work on the cytology of triploids has been productive of variable and, in the main, vague results. Geerts (1911) and van Overeem (1922) describe seven bivalents and seven univalents at the heterotypic metaphase, this being no doubt a reflection of the *Drosera* type of meiosis in a hybrid; their illustrations, too, are faulty, for van Overeem gives none at all for the critical stages, and Geerts' two figures show little that is convincing.

Other workers, in contrast to these two, find no paired chromosomes at all (Gates, 1909, 1915, 1923; Håkansson, 1926; Oehlkers, 1929; Hoeppener and Renner, 1929). Håkansson (1926) shows that the free pair of the diploid *Oe. Lamarckiana* forms, in *Oe. Lamarckiana excelsa* (a triploid corresponding to and probably identical with *Oe. Lamarckiana semigigas*), with a third chromosome, a corresponding group of three, and figures a typical ring and rod trivalent; Geerts and van Overeem obviously failed to observe this trivalent group. The behaviour of the remaining eighteen chromosomes was obscure and not made out thoroughly; he says that the greater part of the chromosomes were linked to one another, without, however, forming a regular ring as in *Lamarckiana*; irregularities arose, also, from the presence of pairs of completely homologous chromosomes in the chain.

Hoeppener and Renner (1929) were also able to see the trivalent in their material of (*Lam. gigas × suaveolens*) *semigigas-flava* and of (*Lam. gigas × Lam. nanella*) *semigigas*. The remainder of the chromosomes seemed to be linked, though fractures in the chain were observed; they were uncertain in how far the breaks into smaller pieces were artefacts due to fixation. Oehlkers (1929), in the twin types *semigigas-flava* and *semigigas-albata* from (*suaveolens × Lam. gigas*), also found that the chains were not perfectly continuous.

The work on *semigigas* forms of *Lamarckiana* thus indicates a trivalent corresponding to the ring pair of the diploid, and a group of eighteen chromosomes more or less linked together into a chain probably, however, broken at various points to form short lengths.

OBSERVATIONS.

Redescriptions of the nuclei figured in my previous paper are presented below, together with further examples from the same material showing other chromosomal configurations. 136 nuclei, with fifteen or more chromosomes, at diakinesis have been examined; at least eighteen were complete, and many had eighteen to twenty chromosomes in one section.

The principal types of chromosome configuration which have been found are illustrated in Fig. 1. Fig. 1f was taken from a multipolar spindle stage, but all the rest were selected from nuclei at diakinesis. The chromosomal groupings may be classified into univalents, bivalents, trivalents, quadrivalents[1], etc., according to the number of chromosomes joined together. The rod univalent (Fig. 1a) only has been observed, indicating that the two ends of the chromosome have different attractions,

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[1] These terms are used here with a merely *numerical* significance.

as shown in *Datura* haploids by Belling (1927); univalents in haploid *Oenotheras*, described by Emerson (1929) and by Davis and Kulkarni (1930), are also all of the rod type. Two bivalent types occur in the triploid, the rod (Fig. 1 *b*) and the ring (Fig. 1 *c*) forms. These are all the
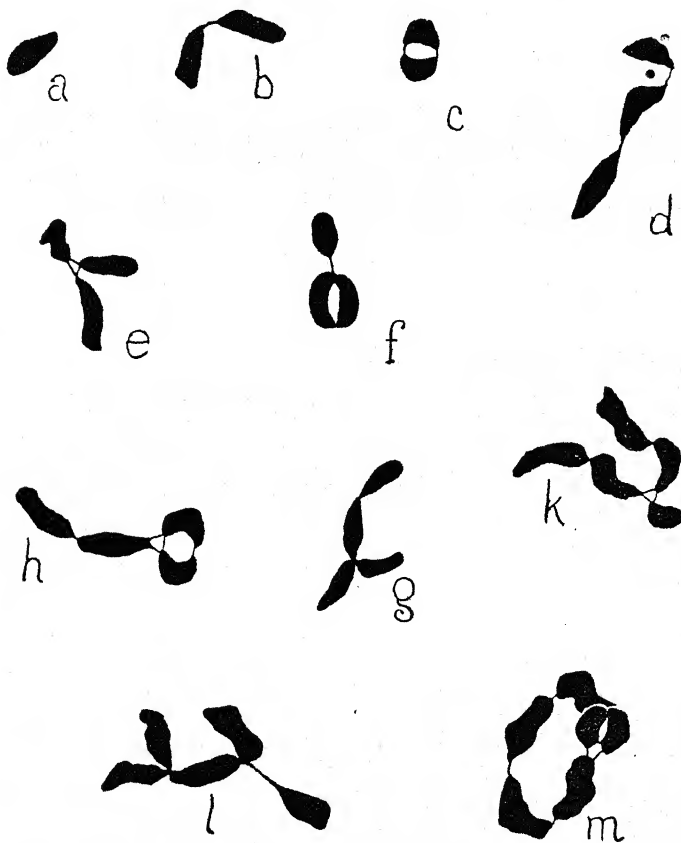
Fig. 1. Types of chromosomal configurations. *a*, Free univalent; *b*, rod bivalent; *c*, ring bivalent; *d*, chain trivalent; *e*, Y-shaped trivalent; *f*, "ring-and-rod" trivalent; *g*, branched chain quadrivalent; *h*, "ring-and-chain" quadrivalent; *k*, branched chain quinquevalent, with triple chiasma at middle of chain; *l*, doubly branched chain or H-shaped quinquevalent; *m*, sexivalent. (For description see text.) × 3300.

possibilities on the assumption that the two ends of any chromosome have specifically different pairing properties. Three forms of trivalent grouping have been found; these are the chain (Fig. 1 *d*), the Y-shaped form (Fig. 1 *e*) and the ring and rod type (Fig. 1 *f*), the last already well figured by Håkansson (1926, Fig. 4 *d*); the Y-trivalent is figured by

Hoeppener and Renner (1929) at Fig. 17 c. Quadrivalent groups are much less frequent; three types of arrangement have been seen, namely, the chain (Figs. 5, 8 f), the association of two rod bivalents by a triple union at the centre of one of them (Fig. 1 g), and the association of a rod bivalent with one end of a ring bivalent by a triple connection (Fig. 1 h). Quinquevalents also occur; the chain form is shown at Fig. 8 f, while Fig. 1 k shows a form made up of two rod bivalents each associated by a free end to the same end of a univalent, thus forming a triple connection at this point; an H-shaped form is shown at Fig. 1 l, and is compounded of two rod bivalents linked at their centres by a rod univalent, this univalent thus having a triple union at each end. All these types of grouping, as will be seen later on, are explicable on the basis of the formula previously ascribed to this triploid. Only one group, a sexivalent, has been found that might form a possible exception; it is shown at Fig. 1 m, and is thought to consist of a ring pair forming, with a chain of four other chromosomes, a branched ring of six; one of the triple connections is obvious, the other more obscure and doubtfully present, since it is in polar view.

The chromosome groups actually found are thus very various and numerous, and only some of the possible arrangements are represented in each nucleus. Fig. 2 is of a whole nucleus at diakinesis, in which are shown four ring pairs, two rod pairs, three free and unattached chromosomes, one Y-shaped trivalent, and one chain of three. Thus, in this case, the longest chain in the nucleus is of three chromosomes only. The Y-shaped trivalent and the chain of three (both on the right-hand side of the nucleus) are not connected, though a rather cursory examination might lead one to suppose a connection, since the adjacent chromosomes of the two sets lie in one focal plane at their nearest points to each other. The nucleolus lies in an upper focal plane, is quite pale staining, and is indicated in outline only as all the chromosomes in its region are below it.

In other nuclei, a high degree of chain formation may be observed; for example, Fig. 3 shows a nucleus at diakinesis in which may be seen five free unattached chromosomes, one chain of six and one group of ten chromosomes, eight of which are joined end to end into a chain, the other two forming a triple junction at one end of the chain. The nucleolus is relatively small, is pale staining and lies in a plane above the chromosome drawn below it. The three free chromosomes at one o'clock are grouped together rather closely, but no connections could be seen between them; moreover their axes are inclined in planes which are opposed in a manner that must dispose of any suggestion of a possible

connection between them. It is to be noticed that when long chains are present in a nucleus it is usual to have a relatively large number of free chromosomes present; in this case there are five.

A somewhat detailed account of the re-examination of the nucleus, previously shown at Fig. 36 (Catcheside, 1930), would seem desirable. A drawing, representing the new interpretation of this nucleus, is reproduced at Fig. 4. There are shown three ring pairs, two of them free from the rest of the chromosomes, and the third (on the left) attached
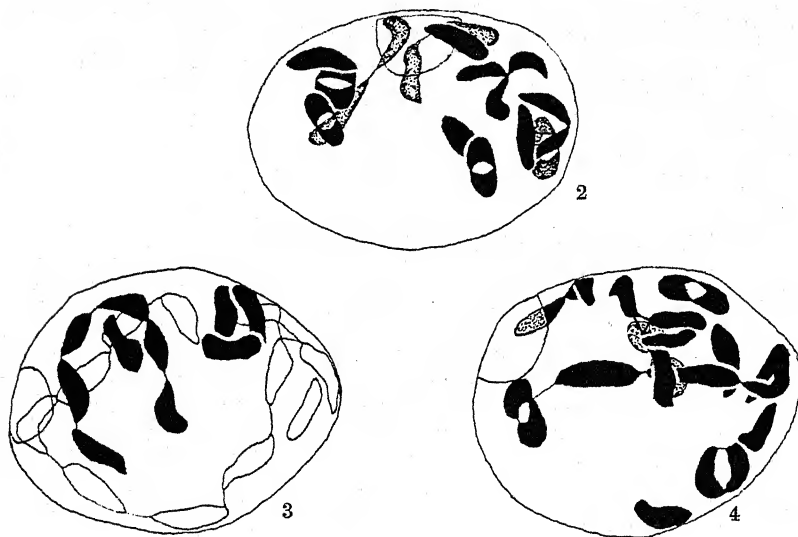
Figs. 2–4. Whole nuclei at diakinesis showing the various chromosome associations. × 3300. Fig. 4 is from the same nucleus as Fig. 36 of Catcheside (1930).

to the end of a chain of four chromosomes; the thread connecting the chain to the ring pair is rather broad and indefinite, suggesting a double structure that cannot be resolved. Above the centre of the nucleus, at 12 o'clock, is shown a triple linkage; previously, the right-hand chromosome of the three was thought to be joined to the single one, lying in a slightly higher focus; this typical Y-shaped trivalent is figured separately at Fig. 1 *e*. Most of the other linkages, suggested in the earlier account, were based upon the shapes of the chromosomes, certain terminations being bent round towards other chromosomes in a manner highly suggestive of a definite thread-like connection. I am now of the opinion that, instead of showing a closed circle of twenty-one chromosomes, this nucleus shows various chromosomal configurations in agreement with

and susceptible of explanation upon Darlington's structural hybridity theory of the Oenotheran chromosome complement. There are present four free unattached chromosomes, two of them at 3 and 5 o'clock, respectively, close to the periphery, one adjacent to the triple union of the Y-trivalent, and the fourth underlying the branched chain of six and inclined near to the right-hand chromosome of the Y-trivalent. The two ring pairs are placed, one of them at shortly after 12 o'clock and the other at about 4 o'clock. At 11 o'clock is a rod pair with the right-hand member bent round in a manner suggestive of a connection with the upper chromosome of the Y-trivalent; no connection can, however, be seen. The other rod pair lies in a deep focus below the Y-trivalent.

The chromosomal configurations shown at later stages, particularly at the heterotypic metaphase, have been studied and all of the fundamental types and most of the important variants from these types have been traced; but definite conclusions are often rendered difficult by the close grouping of the large number of chromosomes about the equator of the spindle. Fig. 5 is a drawing of a bipolar spindle, at the metaphase of the heterotypic division, which appears rather obliquely in profile in the preparation; the chromosomes have been represented in their separate groups. In passing, it may be noted that the few perfectly polar views that have been observed all show the groups of chromosomes more or less evenly spread over the equatorial plane of the spindle. This metaphase shows two ring pairs, two rod pairs, three free chromosomes, one chain of four, and a branched chain of six chromosomes; there is no clear indication, in some cases here, to which pole of the spindle a particular chromosome will pass eventually; possibly this spindle is at a rather early stage before all the spindle fibre attachments have been established.

In Fig. 6 is shown an early anaphase complement of nineteen, two chromosomes appearing in the next section; in this drawing the chromosomes are spread out laterally to some extent into three groups. Here is well shown, on the left, the bent appearance of the chromosomes forming a rod pair; this is due to the spindle fibre attachment being situated approximately at the centre of the chromosome, the free end thus tending to lag in the viscous medium of the spindle. Next to this rod pair is a V-shaped trivalent (a later stage of the straight trivalent of the diakinetic phase), the end chromosomes of which are attached to spindle fibres from the same pole and have a bent shape. There are three other rod bivalents and one free chromosome, and the remainder of the chromosomes are arranged as a branched chain. The arrangement in this case shows little evidence of an alternating zigzag, and, in fact, in the case of

two chromosomes in the chain it is quite impossible to decide to which pole they will ultimately pass.

Fig. 37 of my former paper has been redrawn at Figs. 7 and 8 *a–g*, the latter showing the component associations, seven in all, drawn
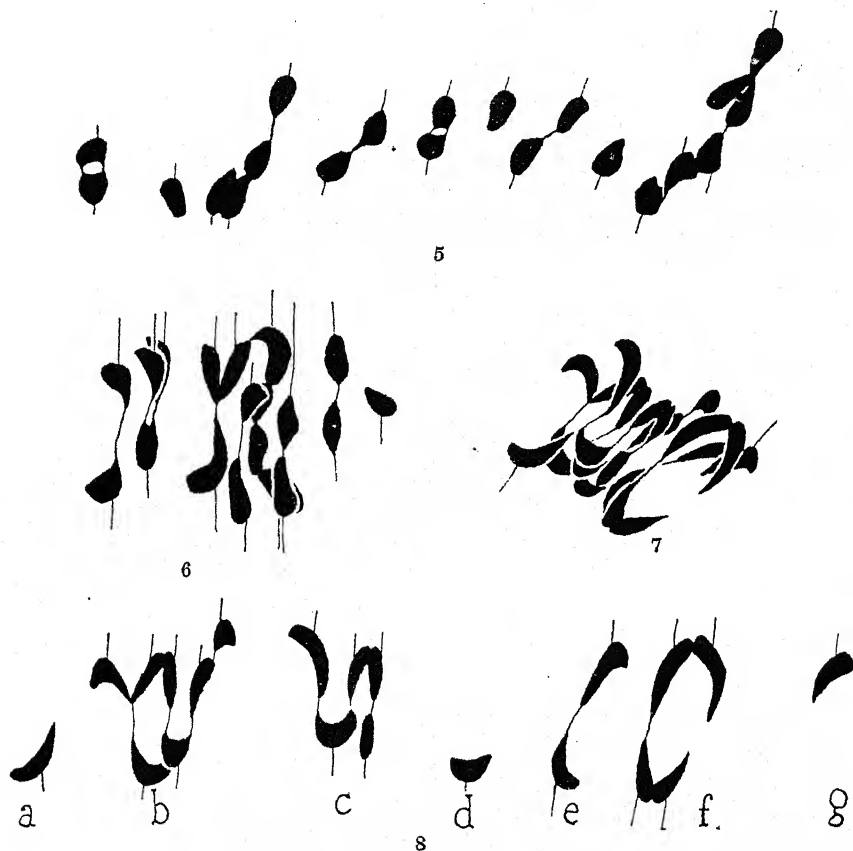
Figs. 5–8. Arrangement of the chromosomal associations at the metaphase or early anaphase of the heterotypic division. × 3300. Figs. 5, 7 and 8 show twenty-one chromosomes each; Fig. 6 shows nineteen present. Fig. 8 is an analysis of Fig. 7, and both are from the same cell as fig. 37 of Catcheside (1930).

separately from one another. I have now represented twenty-one chromosomes; study of the two adjacent sections failed to reveal the three missing chromosomes, and subsequently it was noticed that certain of the chromosomes, looking rather larger and a little clumsy and bent rather sharply at the middle, had a small constriction at this point. These three bodies are evidently each two chromosomes very closely adhering

at their ends; they are (i) the two to the right of the triple union in Fig. 8 b, (ii) the two at the top to the right of the group in Fig. 8 c, and (iii) the two at the bottom of the group in Fig. 8 f. Certain connections previously thought to exist, chiefly on the ground of chromosome shape and the direction of the bent ends, are realised now not to be present; the cause, of the bent chromosomes giving the appearance of stresses in directions likely to suggest actual connections between certain chromosomes, is of course due to the approximately median spindle-fibre connection, together with the free ends lagging in the viscous substance of the spindle. The sharply pointed free ends of certain chromosomes, however, would seem to suggest other possible connections: namely, of the chromosome shown at Fig. 8 a to the connection joining the two last chromosomes on the left of Fig. 8 c, of the chromosome in Fig. 8 g to some indeterminable point, and in Fig. 8 f the possibility of a closed circle of four, a possibility, however, incompatible with the suggested segmental formula, unless there has been a further interchange. The early anaphase under discussion is constituted, then, of three free chromosomes (Fig. 8 a, d, g) which will move freely to one or the other pole; in no case has equatorial splitting of these free chromosomes been seen, such as is described in the case of such univalent members of the complement in species hybrids in certain other genera. There is also a rod pair (Fig. 8 e) with its free ends bent round in the characteristic manner. Fig. 8 b shows a branched chain of seven, with the triple union in the subterminal position, and showing a more or less definitely zigzag arrangement of the chromosomes of the chain; Fig. 8 c shows a chain of five which has some tendency towards a regularly alternate arrangement, while Fig. 8 f is quite peculiar, being a chain of four in which each of the two pairs of adjacent chromosomes are destined to pass to the same pole. This nucleus thus shows four cases in which two adjacent chromosomes must pass to the same pole at the anaphase; non-disjunction is therefore probably more frequent than in the diploid.

Disjunction and disjunctional numbers in triploids have been described and discussed fully by other writers, notably Gates (1909); the present case confirms the general account of anaphase, particularly with reference to lagging of chromosomes, omission of certain laggards from the interkinetic nuclei, the usual ten-eleven disjunctional numbers, and the rarer twelve-nine distribution. In point of interest, it is worthy of note that Fig. 7 (the early anaphase previously described) shows that in this example a twelve-nine anaphasic disjunction is inevitable.

DISCUSSION.

The presence of the various chromosomal configurations, at the heterotypic metaphase and diakinesis, must obviously point to the presence of some noteworthy and as yet unknown peculiarities during the early and middle stages of prophase of meiosis in trisomic and polyploid *Oenothera*. An attempt was made, without success, upon the present material to discover the earlier histories of these several types of chromosome grouping; unfortunately, the open spireme (leptotene?) stages are not satisfactorily stained for the purpose of such detailed study. At the pachytene, too, the central tangled mass was found to be too dense to permit of good resolution.

On the basis of the closed ring of twenty-one chromosomes previously considered to be present, I discussed the question of the syndesis of the chromosomes in *Oenothera*, the obvious thesis being the supposedly indubitable telosynaptic interpretation that had to be placed upon the facts shown by the cytology of the diploid forms. The position of telosynapsis was assailed by Darlington, basing an interpretation of ring formation in *Oenothera* upon structural hybridity, due to segmental interchange between non-homologous chromosomes of the same complex, the chromosomes then being associated by chiasmata, which become terminal at metaphase, with one end of each of two other chromosomes. It is most certainly an hypothesis that will account for a large body of facts, both genetical and cytological; certain older observations seem to be at variance with the requirements of the theory, and need reinvestigation. In particular, these are the early prophase behaviour of the chromosomes, the question of the continuity of the spireme in forms having one or more pairs of chromosomes, and the manner in which the rings and pairs arise, especially whether the pairs are cut off from the pachynema as has been described in various cases.

Before proceeding further with general topics, it must first be clearly indicated that, in view of the facts disclosed by more enlightened study of the triploid of *Oe. pycnocarpa* and presented above in some detail, it is necessary to reverse certain conclusions arrived at in the former paper. In the first place, the chromosomal configurations are fully accounted for on the basis of Darlington's scheme, using the following type of segmental formula:

(a)	AB	CD	EF	GH	KL	MN	OP
(a)	AB	CD	EF	GH	KL	MN	OP
(b)		BC	DE	FG	HK	LM	NO	PA

The two segmental complex formulae (*a*) and (*b*) are taken to represent the complexes of ordinary diploid *Oe. pycnocarpa*, this having a ring of fourteen chromosomes. In the triploid, one of the complexes, let us suppose (*a*), is present in duplicate. If all the possible associations were established (by chiasmata) and maintained until diakinesis, the configuration would be a ring of ring pairs and single rod chromosomes joined together alternately (Fig. 9). All the configurations found at diakinesis or the heterotypic metaphase can be derived from this structure by eliminating various chiasmata. No chromosome arrange-
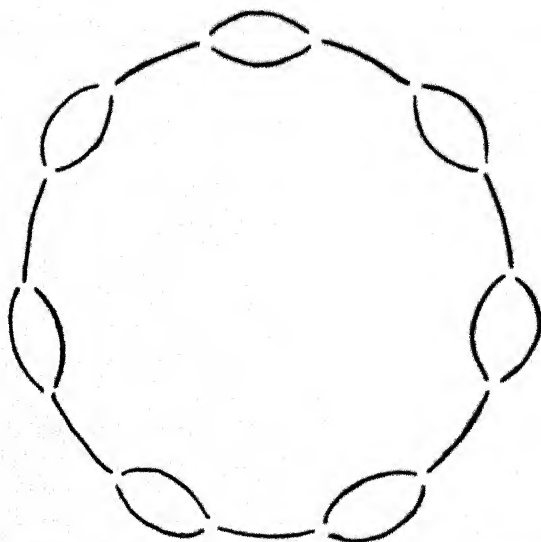
Fig. 9. Diagram of ring of alternate rod chromosomes and ring bivalents, such as would be formed were all the possible metaphase chiasmata established and maintained.

ments, with one doubtful exception (Fig. 1 *m*), have been found which do not fit into this scheme. Certain of the associations of three and of four chromosomes observed in *Datura* (Belling, 1927), *Hyacinthus* and *Tulipa* triploids (Newton and Darlington, 1929) and *Primula* tetraploids (Darlington, 1930 *b*) are not to be expected if the segmental formula proposed is of the correct type; these particular associations have not been found. Moreover, the configurations with triple unions (chiasmata) are not compatible with the theory of segmentation of a continuous spireme. The configurations obtainable on the basis of the segmental formula, together with those not to be expected, are shown diagrammatically in Fig. 10; an asterisk indicates those actually found in the present case. The chromosomal structure, an hexivalent, figured at Fig. 1 *m*, is the

only possible exception seen, and this could be regarded equally as evidence of a new segmental interchange in this material.

Blakeslee and Cleland (1930) have evolved segmental formulae for certain Oenotheran complexes (in those quoted below, letters have been substituted for the figures used by these authors, as being easier to handle

Possible associations.   Impossible associations.
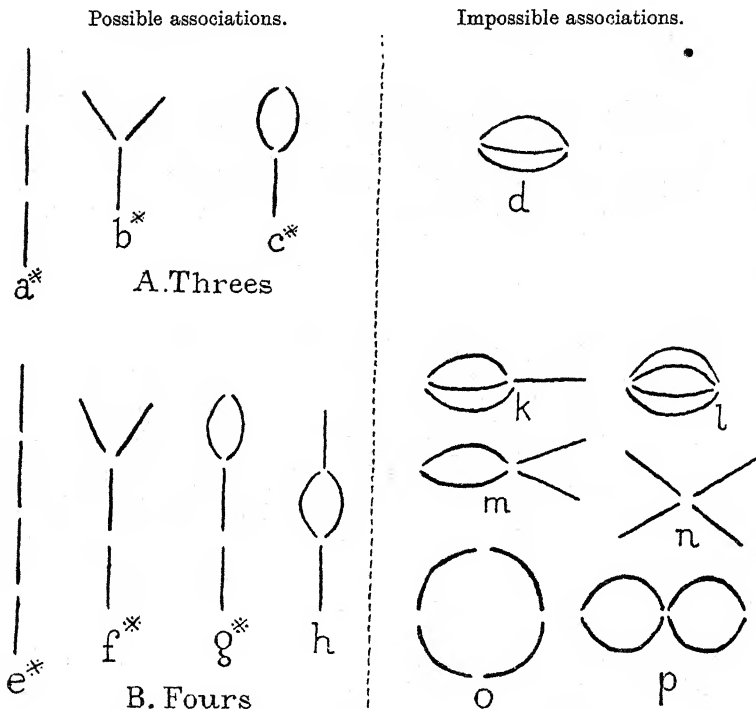
Fig. 10. Diagram of the associations of three and of four chromosomes possible and also impossible on the basis of the segmental formula proposed, together with an indication by an asterisk of those actually found in the present case.

and requiring no point between the two figures). They commence with the completely homozygous *Oe. Hookeri*, this species consisting of two *Hookeri* complexes:

$$AB \quad CD \quad EF \quad GH \quad KL \quad MN \quad OP$$

The complexes of *Oe. Lamarckiana* are made up as follows:

| velans | AB | CD | EH | GF | KL | MN | OP |
| gaudens | AB | CP | EF | GD | NL | MH | OK |

while the complexes of *Oe. suaveolens* are:

| flavens | AD | CB | EF | GH | KL | MN | OP |
| albicans | AD | CE | FH | GL | KN | MP | OB |

Crosses between *Lamarckiana gigas* and *suaveolens* result in the twin types *semigigas-flava* and *semigigas albata* in the F_1 progeny (Hoeppener and Renner, 1929; Oehlkers, 1929). The segmental formulae of these would seem to be:

semigigas-flava	AB	CD	EH	GF	KL	MN	OP
(*velans, gaudens, flavens*)	AB	CP	EF	GD	NL	MH	OK
	AD	CB	EF	GH	KL	MN	OP

and

semigigas-albata	AB	CD	EH	GF	KL	MN	OP
(*velans, gaudens, albicans*)	AB	CP	EF	GD	NL	MH	OK
	AD	CE	FH	GL	KN	MP	OB

It is obvious that both these are completely triploid with regard to the homologous end segments of the several chromosomes; moreover, all of the types of chromosome association found in the *pycnocarpa* triploid will be possible, though the frequencies will be different owing to the lesser number of completely homologous chromosomes in these forms. Indications of the associations expected in the triploids may be seen in the drawings of previous authors. Hoeppener and Renner's (1929) Fig. 16 *a* shows a distinct Y-shaped trivalent, a straight trivalent and an indication of a branched chain of at least five or six chromosomes; their Fig. 17 *a* shows a rod pair, a chain of at least four, a recognisable triple union in a branched chain of six, and one or two free univalents; Fig. 17 *b* shows three fairly definite rod pairs, a Y-shaped trivalent and one free chromosome, but the arrangement of the remainder of the chromosomes in the nucleus is not clear; their Fig. 17 *c* is a Y-trivalent entirely comparable with my Fig. 1 *e*, except that the connecting threads are short to vanishing point.

Håkansson's (1926) drawings of the critical stages in the triploid *Oe. Lamarckiana excelsa* are difficult to make out, but some indications of the configurations expected may be traced. He figures three whole nuclei at diakinesis; his Fig. 4 *a* shows a chain of four, another chain of four with a ring pair possibly attached, and the suggestion of one triple union; Fig. 4 *b* shows a Y-trivalent, a chain of six, a rod pair, a ring pair, and one doubtful univalent; Fig. 4 *c* shows a ring pair and slight evidence of a triple connection. The remainder of the chromosomes, in each of these three nuclei, are too confused to allow of possible interpretation. His Fig. 4 *d* shows an excellent "ring-and-rod" trivalent drawn separately.

These enumerations would indicate, then, that more detailed observations upon all *Oenothera* triploids would reveal much the same types of chromosome associations, though there would be some variations consequent upon the parentage and different segmental constitutions of

different triploid forms. Consideration of this is best left until further studies of triploids and of tetraploids have been undertaken.

The new interpretations in this triploid do not accord with the expectations based upon Sheffield's (1929) hypothesis that ring-formation is a phenomenon governed by genes, and, therefore, this triploid can no longer be held to support a theory based upon ideas of Mendelian inheritance of the ring-forming propensity. Cleland's original theory would thus appear to be a statement of fact, namely that ring-formation is consequent upon heterozygosity of the diploid complement following crossing. But this "theory" does not explain the reason for the coherence of the chromosomes by their ends, since heterozygosity of the diploid complement in practically all other species crosses does not lead to ring-formation, but merely to a failure of pairing or association of any kind and o greater or less sterility. The maintenance of the ring in *Oenothera* species, by the operation of a system of balanced lethals, certainly results in the production of bad pollen and a reduction in seed-fertility in some cases; but even so the seeds are more than sufficient in quantity to maintain the species in abundance. Segmental interchange, then, does not reduce the fertility of the species in diploid *Oenotheras*, though tetraploids and especially triploids are very markedly sterile. The only case, outside *Oenothera*, in which production of chains would seem to be the result of species crosses is in Håkansson's (1925) *Godetia* hybrids; in this genus, again, closely related as it is to *Oenothera*, it is not unlikely that different species differ in their segmental constitutions. Further experiments with *Godetia* species would undoubtedly yield interesting results.

Rhoeo discolor Hance is a plant exactly like Oenotheran forms in its chromosome behaviour at meiosis. It has twelve somatic chromosomes, the attachment constrictions being approximately median except in four chromosomes in which the smaller of the two segments is about half the length of the longer (Darlington, 1929); Katô (1930) refers to these chromosome types as isobrachial and heterobrachial respectively, and has extended Darlington's observations on the behaviour of the ring at the heterotypic division, claiming that the heterobrachial chromosomes have constant positions in the ring, the two pairs being separated on each side by four isobrachial chromosomes. The heterobrachial chromosomes, in rings taking up a regular zigzag arrangement at the metaphase, may be conjoined by their proximal ends (the ends near to which the spindle fibres are inserted), or by their distal ends, but never so that one distal end is attached to one proximal end; moreover, the mode of attachment, by conjunction of either the proximal or the distal ends, is the same in

both pairs of the heterobrachial chromosomes of the same ring. Katô is led to regard the ring in this plant as the outcome of the opening out of a double chain formed by parasyndesis of two spiremes, shown by Kihara in *Rumex acetosella* (1927), the arrangement being such that the two pairs of heterobrachial chromosomes occupy the middle position on the double chain; the two plants, however, are essentially distinct, since *Rumex acetosella* is almost certainly a polyploid.

Darlington (1929 *a*, *b*) has interpreted the ring in *Rhoeo* on his segmental interchange hypothesis; Katô points out that in this event, the heterobrachial chromosomes should conjoin with each other only in a definite mode at their proximal or their distal ends, and not in either mode, as he had shown. Katô makes no mention of the ring ever opening out to form a chain, nor of the latter breaking into two or three shorter chains; this, of course, may be a climatic difference. In the condensed chromosomes at the metaphase it would be very difficult to pick out the heterobrachial chromosomes for certain, and to determine their exact mode of attachment; at any rate the constant position within the circle is not contrary to the requirements of Darlington's theory, and the other observations are so difficult as to be of doubtful worth. (Cf. illustrations of Belling, 1927; Darlington, 1929 *b*; and of Katô, 1930.)

Triple junctions (chiasmata) are a marked feature of this triploid, though it is often difficult to observe the connecting threads owing to their shortness. The triple junction may be present alone, either as a Y-shaped group with the triple union at the angle of the Y, or in a ring and rod trivalent; no case has been observed in this material in which the rod chromosome has closed round to take part in a triple chiasma at the other side of the ring as well, and in fact the absence of such a configuration is in agreement with the segmental formula, as shown in Fig. 10 *d*. Such structures might be seen in trisomic mutants of *Oe. Lamarckiana* in which the extra chromosome was one of the free pair. A trivalent of this type should also be obtainable in *Oe. Lamarckiana semigigas*, but has not been observed as yet. The triple junction may also form part of a branched chain, as shown above in various cases.

The demonstration of the three connecting threads in the triple chiasma proves several things about the Oenotheran chromosome association, namely (i) that already at diakinesis the chromosome is split longitudinally into two chromatids, as in other organisms with parasynapsis, (ii) that there is an exchange of partners between all three of the mating chromosomes, for otherwise the three connecting threads would not be observed, (iii) that the chiasma is subterminal or "terminal,"

there being a very minute portion of the chromosome, distal to the chiasma, forming, with the corresponding portion of the mating chromatid, the connection (Fig. 11 *b*). This would indicate that the connecting thread in a chain is double, from the standpoint of the chromatids (Fig. 11 *a*). Normally, this doubleness is not resolvable owing to the close adherence of the chromatids in the chromosome. In the trivalents the pairing portions of the several chromatids are often shown separately, but in certain cases—frequently in ring and rod forms—one of the connections becomes very short and then the other two merge and cannot
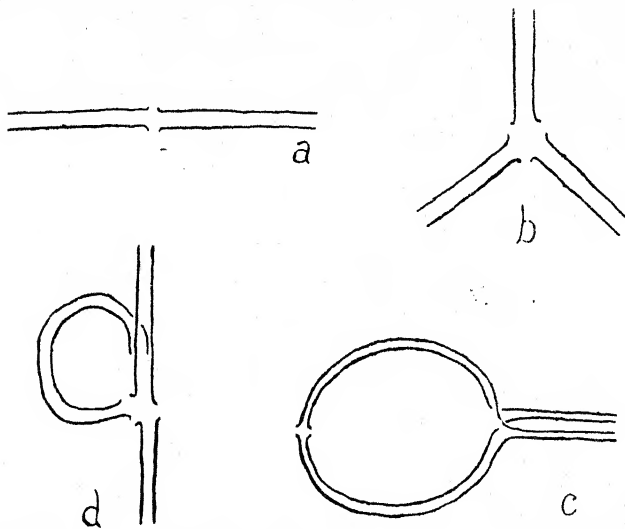
Fig. 11. Diagrams of: *a*, exchanges between two pairs of chromatids, as in a chain connection; *b*, exchanges between three pairs of chromatids, as in a triple chiasma; *c*, ring pair with an interstitial chiasma involved; *d*, interstitial association in a trivalent, possibly the result of reduplication following inversion of a chromosome portion, as figured by Håkansson (1930) at Fig. 3 *o* in a secondary of *Oe.* mut. *cana*.

be distinguished from one another owing to the close adherence. This shows that the connection, in a chain or ring or other configuration, between two chromosomes, must be double, appearing single owing to the close approximation of the two paired portions.

In the hypothetical configuration given above, where all the possible chiasmata between all three of fourteen segment types (*A–P*) have been established, the number of these is twenty-eight; that is the minimum number of prophase chiasmata that would give this metaphase configuration. The minimum number of chiasmata in the diploid to give a closed ring (or seven pairs) is fourteen; in cases where there is a chain of fourteen

chromosomes, the number of chiasmata is thirteen. The numbers of chiasmata (counting triple chiasmata as derived by terminalisation from two simple ones—shown by Darlington, 1929 *b*, 1930 *b*) actually found in the triploid in uncut nuclei in ten cases counted was always fourteen; seven were at diakinesis, and three at metaphase or early anaphase; Figs. 2, 3, 4, 5 and 7 (or 8) show five of these. This indicates that the most frequent number is exactly the same as in the diploid, a result in agreement with the theories of pairing and chiasma formation in diploids and triploids, and showing that the number of prophase chiasmata in the diploid is little if at all in excess of the number of metaphase chiasmata. In diploids two chromosomes pair throughout the length of their homologous parts (Fig. 12 *a*); in triploids (Fig. 12 *b*) one chromosome may pair
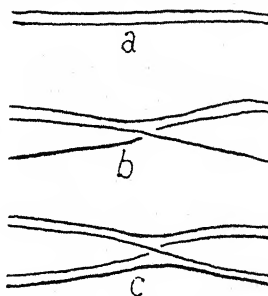
Fig. 12. Diagrams of lateral association of *a*, two, *b*, three, and *c*, four, chromosomes to show that the length of paired homologous chromosomes in the bivalent and trivalent is equal and half the length in the quadrivalent.

with a second in one part of it, and with a third in another part of it, but the total length of paired homologues will be no more than in the diploid, hence there is little chance for the occurrence of more chiasmata in the triploid than in the diploid. A slight increase is possible (though it must be rather infrequent), since the interference may be reduced slightly by one chromosome pairing with two others in different parts of it. The number of metaphase chiasmata in tetraploid forms would be expected to be twenty-eight, or a few more than this, as the length of paired homologous chromosomes is doubled by introduction of a fourth chromosome into the arrangement shown at Fig. 12 *b*, resulting in the arrangement illustrated at Fig. 12 *c*. The number of chiasmata actually found thus agrees with the expectation and forms an added argument in favour of the belief that the pairing between the chromosomes is controlled by the establishment of chiasmata.

In all the cases here figured the chiasmata are terminal, though interstitial chiasmata should be possible in the ring pairs, formed of

chromosomes apparently completely homologous; no cases of interstitial chiasmata were seen, in spite of a special search made for them. Håkansson (1930) figures some good cases of interstitial chiasmata in certain trisomic mutants of *Oe. Lamarckiana*, namely mut. *curta*, a secondary mutant from *cana* with fourteen and a half chromosomes, and mut. *lata*. In the secondary from *cana*, with the extra half chromosome, he figures several singular configurations; one is of a chromosome with a small fragment attached to one end, and he regards this as being a stage in the translocation of a segment from one chromosome to another (his Fig. 3 *p*); another figure (his Fig. 30) shows a free end of one chromosome of a Y-trivalent bent round and attached to another chromosome at a point near to the triple union. Fig. 11 *c* is a diagrammatic representation of a ring bivalent, with interstitial chiasma, as figured by Håkansson (1930). In a ring bivalent, it is probably the case that the two chromosomes are homologous throughout their lengths; at least this must be true in homozygous forms, such as *Hookeri, deserens, blandina* and *purpurata*, and in certain pairs of chromosomes in triploids. Hence it is to be expected that a chiasma may be established at any point in the length of these two chromosomes. A diagrammatic representation of Håkansson's other peculiar chromosome structure is shown at Fig. 11 *d*; this structure may perhaps indicate the occurrence of reduplication following inversion of a chromosome portion at some stage in the history of the nucleus showing this peculiar structure.

In no case, in the literature, have laterally paired threads been observed at the prophase; in diploids, with free pairs, the descriptions state, or lead one to suppose, that these pairs are cut off from a continuous pachynema at, or immediately subsequent to, the second contraction. The early prophase in *Oenothera* is a difficult stage to study, and probably the ideal fixative has as yet to be discovered for this stage, before the secrets of chromosome behaviour at this period will be better understood.

The evidence of this triploid *Oenothera*, then, points to the conclusion that pairing between different chromosomes is consequent upon the establishment of chiasmata in homologous parts of different chromosomes; that, in ring-forming chromosomes, the homologous parts are extremely short and localised at the ends of the chromosomes and so arranged that while one end of a particular chromosome will pair with one end of another chromosome, the other end of the first chromosome will pair with one end of a third chromosome and thus establish a chain. That homologous segments of chromosomes are arranged in this way seems to be indubitable; the question of the agency by which such

arrangements are established will not be dealt with here, except to emphasise that segmental interchange combined with crossing, such as is possible with freedom in the open-pollinated Oenotheras, meets all the requirements (see Darlington, 1929).

SUMMARY.

A corrected account of the critical stages in the cytology of the pollen mother cells of triploid *Oe. pycnocarpa* is given. Various associations of chromosomes occur, including free univalents; rod and ring bivalents; chain, Y-shaped and "ring-and-rod" trivalents; chain, branched chain and "ring-and-chain" quadrivalents; and other associations involving higher numbers of chromosomes, with or without triple chiasmata.

All the types of chromosomal grouping, with one doubtful exception (a sexivalent), conform to the requirements of the segmental formula proposed, whence it must be deduced that structural hybridity forms a correct explanation of the chromosome complement and its behaviour.

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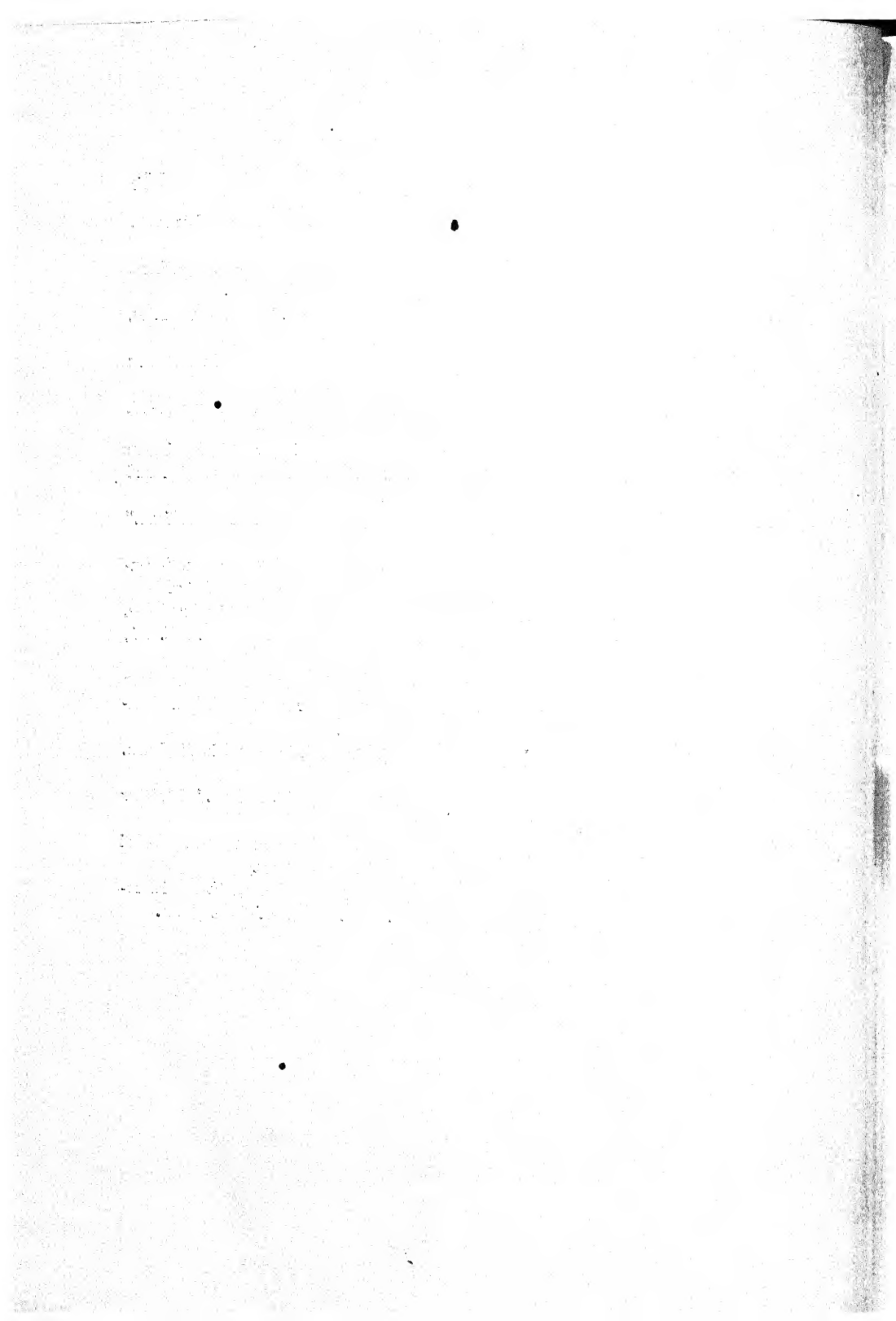
STUDIES ON CERTAIN *PETUNIA* ABERRANTS.

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(With Plate III and Six Text-figures.)

In a recent publication the senior author reported the appearance of a tetraploid plant with 28 somatic chromosomes among the progeny of a plant of a variety of *Petunia violacea* ($n = 7$, $2n = 14$) with blue corolla and white pollen grains (Kostoff, 1930 *b*). This variety had been inbred for two generations without any manifestation of variability among the progeny. The tetraploid plant was characterised by the following features: thick, juicy stems; broadly rounded, succulent leaves, usually sessile; large flowers with a broadly opened corolla whose petals were relatively thick and with a more intense colour in the outer portions; large sepals, about 1 cm. in length; white anthers and pollen. This plant grew much more quickly than its sister plants ($n = 7$, $2n = 14$) and began to flower about 8 days before them. The mother plant and sister plants were self-fertile when pollinated in the bud stage, but the tetraploid was self-sterile in spite of many self-pollinations carried out at various stages of flower development. An examination of the flower buds during the period of meiosis showed that, while in the diploid mother and sister plants this process was entirely normal, in the tetraploid plant meiosis was accompanied by irregularities and the occasional formation of dyads. In the diploid plants there was a relatively low percentage, about 2 per cent., of abortive pollen, while in the tetraploid about 50 per cent. of the pollen grains were found to be abortive. The origin of this plant was attributed to the disturbing influence of a transient cold period upon the meiotic divisions in the mother plant, and the resultant formation and later fusion of gametes with an increased chromosome content.

The tetraploid, though self-sterile, set seeds when pollinated with pollen from a variety of *P. violacea* ($n = 7$, $2n = 14$) with blue pollen. This variety had the following characteristics: thin, dry stems; small, spatulate leaves with relatively long petioles; small flowers with a violet corolla whose petals were thin and most darkly coloured in their basal portions; small, narrow sepals, about 0·5 cm. long; dark blue anthers and pollen. From the seeds produced by this cross 20 hybrid plants were raised. This progeny will be the subject of the present paper, and the

plants will be considered under the following four groups into which they are placed on the basis of their somatic chromosome number: (*a*) tetraploid, with 28 somatic chromosomes (16 plants); (*b*) hypotetraploid, with 27 somatic chromosomes (2 plants); (*c*) triploid, with 21 somatic chromosomes (1 plant); and (*d*) hypotriploid, with 20 somatic chromosomes (1 plant).

TETRAPLOID.

With the exception of a single plant, no. 2, which differed in flower only, the 16 tetraploid plants were quite uniform in habit. They grew rapidly, were the tallest plants (Plate III, fig. 3), and began to flower from a week to 10 days before the other sister plants. The morphological characters in general were intermediate between those of the father and mother plants. The foliage leaves showed some variation in form and size on one and the same plant, but most commonly they were broadly spatulate, the basal leaves were large and with relatively long petioles, the upper ones were small and either sessile or with very short petioles. The flowers were large, the corolla was a deep violet in the bud stage and a lighter violet colour when the flower opened, with the outer portions of the petals crimped and curled slightly backwards (Text-fig. 1 *f*). In size the flowers resembled most closely those of the mother tetraploid plant, but in colour they were intermediate between the two parents. The anthers and pollen were blue in colour, but a very much lighter blue than in the father plant. In the case of the exceptional plant, no. 2, the flower, the only feature in which it differed from its sister plants of this group, was smaller (Text-fig. 1 *e*) and its petals, although of the same colour as the other tetraploids, were much thinner and less crimped and curled. In general it resembled the flower of the father plant, though its colour was intermediate between the two parents, and the pollen and anthers were a lighter blue than those of the father.

In *Nicotiana* the character "blue pollen" has been found to be incompletely dominant (Kostoff, 1930). All the tetraploid plants, as well as the others of this progeny to be considered later, had neither the white pollen of the mother nor the deep blue pollen of the father, but a light blue pollen, a fact that showed them to be truly a cross-product between the tetraploid plant with white pollen and the normal diploid variety with dark blue pollen.

In the somatic metaphases in the root tips of all 16 plants of this tetraploid group, 28 chromosomes were counted (Text-fig. 2). Flower buds of these plants, as well as of the others of the progeny to be considered

later, were studied in temporary aceto-carmine and in permanent preparations. For the latter, material was fixed in a modified Bouin's solution and sections stained in iron-alum haematoxylin. During diakinesis in the pollen mother cells (P.M.C.) of the tetraploids the chromo-
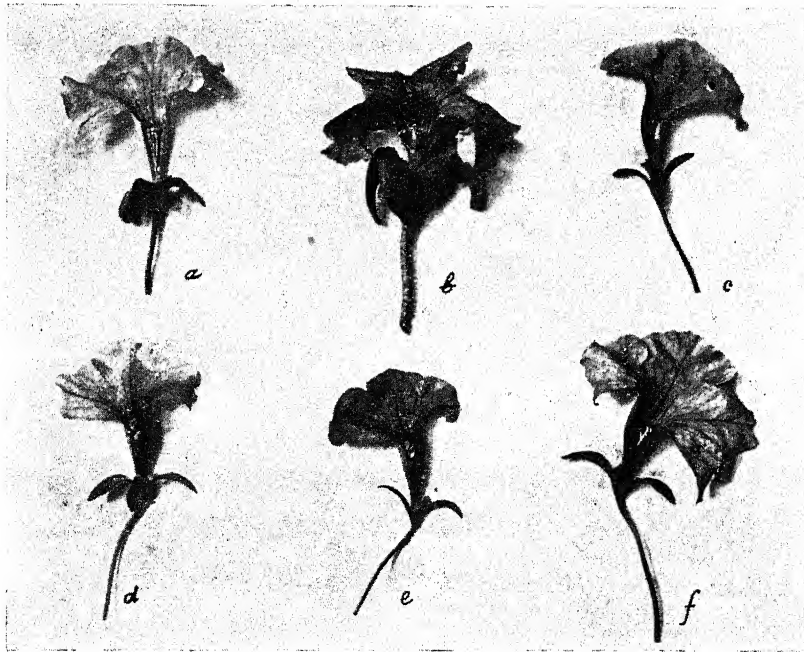
Text-fig. 1. Flowers of representative plants of the progeny: *a*, of the triploid; *b*, of the hypotriploid; *c*, of the hypotetraploid plant no. 16; *d*, of the hypotetraploid plant no. 3; *e*, of the tetraploid plant no. 2; *f*, of the other tetraploid plants.
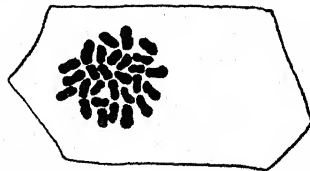
Text-fig. 2. Somatic metaphase with 28 chromosomes from a tetraploid plant.

somes were found associated variously, usually in groups of four as shown in Text-fig. 3 *a*, though associations of two were also found. Belling and Blakeslee (1923) have also reported finding associations of 4 chromosomes during diakinesis in tetraploid Daturas. In the heterotypic metaphase

14 chromosomes (Text-fig. 3 *b*) were observed as in the tetraploid mother plant. The reduction division was observed to proceed quite normally, only occasionally one or two chromosomes were observed leading the advance to the poles in the early heterotypic anaphase (Text-fig. 3 *c*) or lagging on the spindle in the late heterotypic anaphase (Text-fig. 3 *d*). In the homeotypic metaphases 14 chromosomes were usually counted (Text-fig. 3 *e*). As to be expected from such an almost regular meiotic process the pollen formed contained a very low percentage, about 2 per
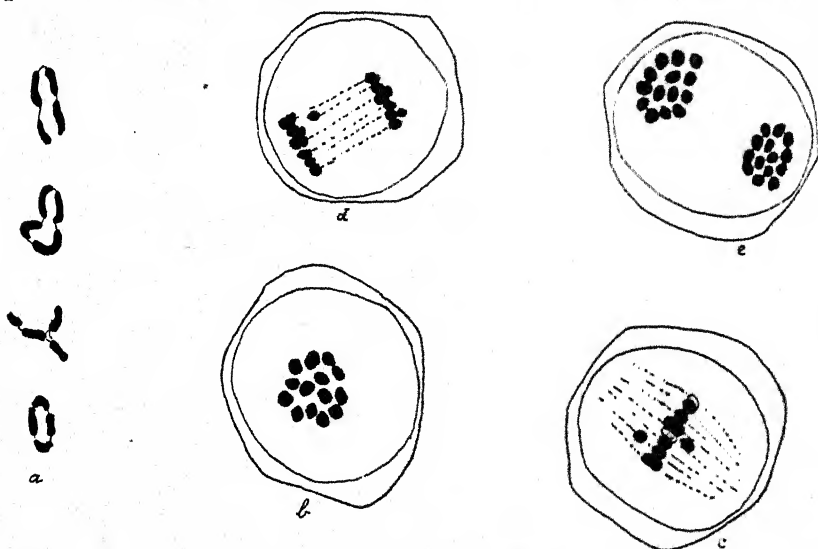
Text-fig. 3. Reduction division in a tetraploid plant. *a*, Chromosomes in groups of fours during the diakinesis; *b*, heterotypic metaphase; *c*, late heterotypic metaphase or early heterotypic anaphase in side view; *d*, late heterotypic anaphase; *e*, homeotypic metaphase.

cent., of abortive grains. This fact is in contrast to the irregularities in meiosis and relatively high percentage, about 50 per cent., of abortive pollen found in the mother plant. In other words, these tetraploid plants of the progeny are cytologically stabilised. Furthermore, these plants all set seed readily when self-pollinated.

The origin of these tetraploid progeny may be explained in two ways. (1) There is the possibility that the egg nucleus ($n = 14$) of the tetraploid mother plant may have been fertilised by both the generative nucleus ($n = 7$) and the vegetative one ($n = 7$) from the pollen grain of the diploid father plant, so that the resultant zygote contained $14 + 7 + 7 = 28$ chromosomes which, by subsequent division, developed the tetraploid progeny. Although it is a debatable question whether more than a single

nucleus ever fuses with the egg nucleus, there seems a possibility in these cases that the 7 unpaired chromosomes of the egg in such an uncomplemented condition following fertilisation with a 7-chromosome generative nucleus might attract the additional 7 chromosomes from the vegetative nucleus of the male contribution as well, and the endosperm develop only from the polar nuclei of the mother plant. That diploid endosperm development can occur from the polar nuclei alone under the stimulative activity of the pollen tube of a foreign species or genera, without any fusion of the vegetative nucleus from the pollen with them, has been reported by Kostoff (1930 a) in the case of certain *Nicotiana* species. In the present instance there are conditions making such a development more probable, *i.e.* each of the polar nuclei of the tetraploid mother plant had 14 chromosomes, so that with their fusion there were 28 chromosomes, while under normal conditions, with the added contribution from the vegetative nucleus of the pollen, in the diploid plants there are only 21 chromosomes present in triploid endosperm (7 + 7 of polar nuclei + 7 of vegetative nucleus = 21). (2) On the other hand, however, it is very probable that the 14-chromosome egg of the tetraploid mother plant was fertilised by a generative nucleus also containing 14 chromosomes, such a generative nucleus originating from a dyad pollen grain. The father plant was observed to form dyad pollen grains when grafted on to *Solanum nigrum* (Kostoff, 1930) and pollen was often taken from flowers of shoots so grafted and used in pollinating the tetraploid plant.

HYPOTETRAPLOID.

The two hypotetraploid plants, no. 3 and no. 16, differed somewhat from each other in some features. Plant no. 3 was almost indistinguishable from the tetraploid plants, it was only slightly slower in growth, was shorter in height, and had a darker green foliage. The foliage leaves were very similar in form and size to the tetraploids, and the flowers were practically inseparable from those described for the 15 tetraploids, if any difference it was in a slightly smaller size (Text-fig. 1 *d*). Plant no. 16 was slower in growth, bloomed about 10 days later, was shorter, and had a less luxuriant foliage than plant no. 3 (Plate III, fig. 2). The foliage was similar in form and size to that of plant no. 3, but much lighter in colour. The flower, as shown in Text-fig. 1 *c*, was intermediate between that of the exceptional tetraploid, plant no. 2 with the father-like flower, and that of the other tetraploids and plant no. 3.

The sections of the root tips of both of the hypotetraploid plants, as

illustrated by the drawing in Text-fig. 4 a, showed in all instances 27 somatic chromosomes in the metaphases. In the flower buds studied
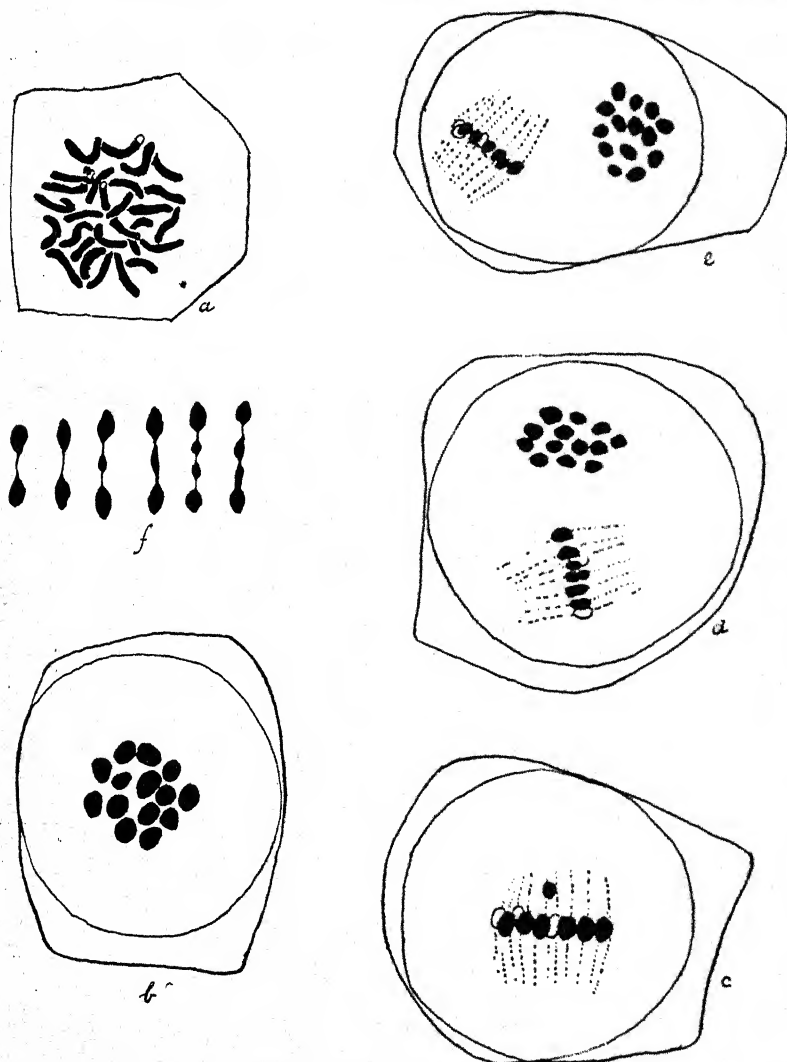
Text-fig. 4. a. Somatic metaphase in the root tip of a hypotetraploid *Petunia* with 27 chromosomes; b–e, reduction division in a hypotetraploid plant; b, heterotypic metaphase; c, late heterotypic metaphase, side view; d, e, homeotypic metaphases with 13 d and 14 e chromosomes; f, types of chromosome separation during the heterotypic and frequently in the homeotypic divisions in all the *Petunia* types studied as well as in the father plant (n = 7), schematically represented.

during the period of meiosis, the chromosomes of the P.M.C. in diakinesis associated in groups of four very much like those observed in the tetra-

ploids, though occasionally associating in threes and appearing singly as in the triploid and hypotriploid described later. In the heterotypic metaphase, 14 chromosomes appeared, as illustrated in Text-fig. 4 b. The meiotic process was accompanied only occasionally by the leading ahead (Text-fig. 4 c) or lagging behind of a single chromosome in the early or late anaphases, irregularities that were observed more frequently during the heterotypic than during the homeotypic divisions. The single chromosome apparently joined one or another of the polar groups, so that at the reduction division the chromosomes were distributed into groups of 13 and 14 (Text-fig. 4 e, d), and pollen grains of these two chromosome numbers should obviously have been formed in equal numbers. As in the case of the tetraploids, the pollen was found to contain a very low percentage (about 2–5 per cent.) of abortive grains, i.e. the pollen grains with 13 chromosomes appeared to be viable as well as those with 14 chromosomes. Both of the hypotetraploid plants were self-fertile and set seed readily when pollinated before the flowers opened.

The origin of the hypotetraploid plants finds a probable explanation in the fusion of a 13-chromosome egg nucleus with a 14-chromosome generative nucleus, since one might expect the formation of egg nuclei with 13 chromosomes in the tetraploid mother plant where the meiotic divisions were irregular. The probable formation of generative nuclei with 14 chromosomes on the part of the diploid father plant has already been pointed out in discussing the origin of the tetraploids. An origin from egg cells with 14 chromosomes and generative nuclei with 13 chromosomes appears less likely, since the tube of such a pollen grain so genetically unbalanced would scarcely reach the ovary. The possibility expressed previously, a fertilisation of the egg nucleus, in this case with 13 chromosomes, with both the generative and vegetative nuclei of the diploid father plant might also be advanced here as a possible explanation of the origin of the hypotetraploid plants.

TRIPLOID.

The single triploid plant, no. 13, grew almost as rapidly as the tetraploids, and began to flower shortly after them. It was more bushy and shorter in its growth, and had a more dense foliage than the tetraploids (Plate III, fig. 4). Otherwise, it showed morphological characters of foliage and flowers (Text-fig. 1 a) similar to those already described for the 15 tetraploid plants, and was practically inseparable from them on such a basis. The anthers and pollen were also of a light blue colour.

Preparations of the root tips showed the somatic number of chromosomes to be 21, as illustrated in the metaphase in Text-fig. 5 *a*.

A study of the flower buds of this plant during meiosis showed that in diakinesis the chromosomes usually appeared associated in threes and twos, occasionally also singly. In the triploid *Datura* Belling and Blakeslee (1922, 1923) observed that during the diakinesis the chromosomes were associated in groups of 3. In the first metaphase of the triploid *Petunia* from 10 to 14, rarely 9, chromosomes were observed and these varied greatly in size. Apparently, univalent, bivalent, and trivalent chromosomes were formed. Partial and complete trivalency in *Nicotiana*, a genus very closely related to *Petunia*, has been reported by Kostoff (1930 *a*); and Nishiyama (1928) has reported complete trivalency in *Lycoris*. It seems that some of the homologous chromosomes form trivalent gemini, though the affinity among the homologous chromosomes is so low that some of them are loosely joined, while others do not form trivalent gemini at all; as a consequence of this trivalents, bivalents, and univalents appear in the heterotypic metaphases (Text-fig. 5 *b*). If all the chromosomes were to form trivalent gemini the formula would be as follows:

$$\frac{\begin{array}{c}a_1\\a_2\\a_3\end{array}}{} + \frac{\begin{array}{c}b_1\\b_2\\b_3\end{array}}{} + \frac{\begin{array}{c}c_1\\c_2\\c_3\end{array}}{} + \frac{\begin{array}{c}d_1\\d_2\\d_3\end{array}}{} + \frac{\begin{array}{c}e_1\\e_2\\e_3\end{array}}{} + \frac{\begin{array}{c}f_1\\f_2\\f_3\end{array}}{} + \frac{\begin{array}{c}g_1\\g_2\\g_3\end{array}}{} = 7 \text{ chromosomes,}$$

and in the heterotypic metaphase there would appear the 7 trivalent chromosomes *a*, *b*, *c*, *d*, *e*, *f*, *g*, according to the scheme in *Lycoris*. In the case of the triploid *Petunia* all of the chromosomes do not seem to be so tightly joined as to give a plate with 7 chromosomes, and such plates are formed as those illustrated in Text-fig. 5 *b* whose formula will be:

$$\frac{\begin{array}{c}a_1\\a_2\\a_3\end{array}}{} + \frac{\begin{array}{c}b_1\\b_2\\b_3\end{array}}{} + \frac{c_1}{c_2} + \frac{d_1}{d_2} + \frac{e_1}{e_2} + \frac{f_1}{f_2} + \frac{g_1}{g_2} + c_3 + d_3 + e_3 + f_3 + g_3 \quad 12 \text{ chromosomes,}$$

where altogether 12 chromosomes appear. Recently, Yarnell (1929) has reported what he deemed to be pairing of non-homologous chromosomes in a triploid *Fragaria* with 21 somatic chromosomes, which led him to point out the disharmony between his case and a fundamental tenet of Mendelism. If we understand his case and his interpretation, the formula in the triploid *Fragaria* according to the above scheme would be

$$\frac{a_1}{a_2} + \frac{b_1}{b_2} + \frac{c_1}{c_2} + \frac{d_1}{d_2} + \frac{e_1}{e_2} + \frac{f_1}{f_2} + \frac{g_1}{g_2} + \frac{a_3}{a_2} + \frac{c_3}{b_2} + \frac{e_3}{d_2} + g_3 = 11 \text{ chromosomes,}$$
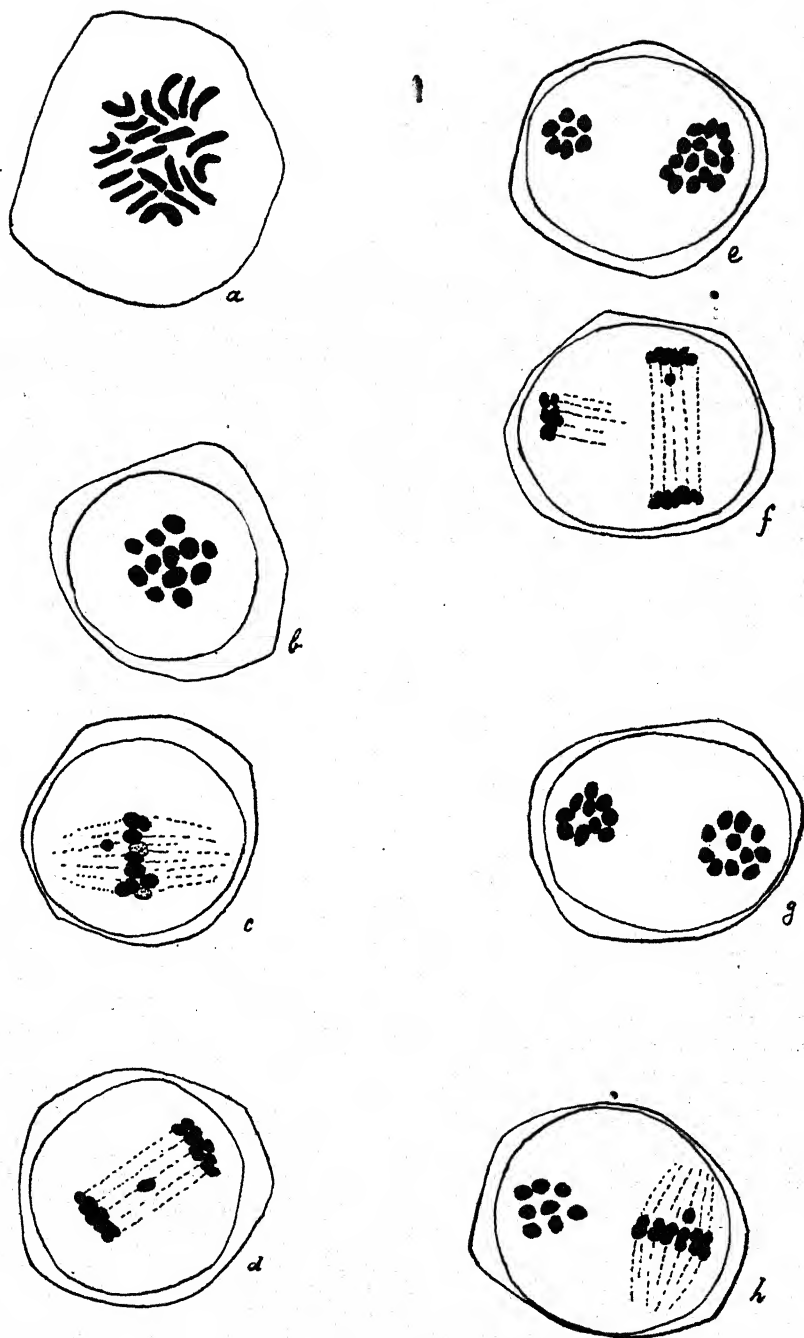
Text-fig. 5. *a*. Somatic metaphase in the root tip of the triploid *Petunia*. *b–h*. reduction in
the triploid *Petunia*: *b*. heterotypic...

to account for the 11 chromosomes he observed most frequently in the P.M.C. at first metaphase and for the pairing of non-homologous chromosomes. We have also sometimes observed metaphases in the triploid *Petunia*, which has the same number of somatic chromosomes as the triploid *Fragaria*, in which there were 11 chromosomes, but we are inclined to believe that the chromosomes are arranged not as 10 bivalents and a single unpaired chromosome but according to the following formula:

$$\frac{a_1}{\frac{a_2}{a_3}} + \frac{b_1}{\frac{b_2}{b_3}} + \frac{c_1}{\frac{c_2}{c_3}} + \frac{d_1}{d_2} + \frac{e_1}{e_2} + \frac{f_1}{f_2} + \frac{g_1}{g_2} + d_3 + e_3 + f_3 + g_3 = 11 \text{ chromosomes.}$$

This appears more probable to us for our case, since we cannot identify individual chromosomes either in diakinesis or early anaphase. Fixation, imbedding, and staining are processes that might partially affect the apparent size and shape of the chromosomes, so that judgments concerning their size and shape must be considered as something very relative, especially during the diakinesis, when it depends very much on their position in the preparation also—whether they are parallel or perpendicular to the slides. If one judges valency of the chromosomes from the figures that appear during the early anaphase where 2, 3, or 4 chromosomes might be observed as illustrated in Text-fig. 4 *f*, one might draw an erroneous conclusion, since all these figures found here in the case of the triploid are also found in pure species with normal diploid chromosome content where only bivalent chromosomes are present; as well as in crosses where one might expect univalent, bivalent, and trivalent chromosomes (Kostoff, 1930 *a*). Even in the case of an androgenic haploid *Nicotiana* (Kostoff, 1929), such figures have been observed. It does not seem necessary, therefore, on the basis of the morphology of the chromosomes, to assume in such cases that there is a pairing of non-homologous chromosomes and a disharmony with a fundamental tenet of Mendelism.

The heterotypic division in the triploid *Petunia* was irregular, single chromosomes leading the advance to the poles or lagging on the spindle (Text-fig. 5 *c, d*) were often observed, and the variation in the distribution of the chromosomes was especially noticeable. As a result of the varying distribution in the heterotypic division there were observed the following numbers of chromosomes in the two metaphase plates of the homeotypic division: 7 and 14, 8 and 13, 9 and 12, 10 and 11 (Text-fig. 5 *g, h*). Most frequently, however, 10 and 11, and 9 and 12 chromosomes were counted. In a single instance 7 and 15 chromosomes (Text-fig. 5 *e*) were counted

in the two plates of the homeotypic metaphase, *i.e.* a univalent had divided. Occasional divisions of univalent chromosomes were observed in the androgenic *Nicotiana* haploid (Kostoff, 1929) and in the hybrids *N. Tabacum* × *N. glauca* (Kostoff, 1930 *a*).

The homeotypic division was more regular than the heterotypic one, and the chromosomes leading the advance to the poles or lagging on the spindle (Text-fig. 5 *f*, *h*) were not observed so often as during the preceding division. As a result of such irregularities of chromosome distribution, non-disjunctions, and lagging of the chromosomes on the spindle, a high percentage, about 40–50 per cent., of the pollen formed was abortive.

The triploid plant obviously originated from the fusion of the 14-chromosome maternal gamete of the tetraploid plant with a 7-chromosome gamete from the diploid paternal plant.

The triploid plant was found to be self-sterile, and all attempts to obtain seeds by self-pollination in various stages were unsuccessful. It set seed, however, when pollinated by pollen from the tetraploids or hypotetraploids.

HYPOTRIPLOID.

One plant, no. 1, was outstanding, not only because of being the slowest growing of the entire progeny and flowering two weeks after all its sister plants, but because of its markedly different habit (Plate III, fig. 1), which reminded one of the mother plant. The stems were much thicker and juicier than those of the sister plants. The foliage leaves were very broad, almost sessile, and lighter in colour than those of any of the other plants. They were not only much larger but, instead of being more of less flatly extended, as those of the others, were wrinkled and curled. The sepals, like the foliage leaves, were very large and curled (Text-fig. 1 *b*). The flowers were larger and heavier than those of any of the sister plants; the petals were more darkly coloured, and showed the same tendency to curl already noted in the foliage leaves (Text-fig. 1 *b*). From the upper central portion of the anthers there developed invariably small leaflets coloured slightly less darkly than the petals, and the anthers opened on either side of this leaflet to display a small amount of pollen a lighter blue in colour than that of the other plants. The heavy stem and large foliage leaves of this plant resembled those of the mother plant, but the slow growth, the large, much-curled flowers, curled foliage, and blue pollen, were not like those of the maternal tetraploid plant. In general appearance it recalls those plants obtained by Stein

(1926) after irradiation, an appearance giving the impression of inhibition and distortion of growth.
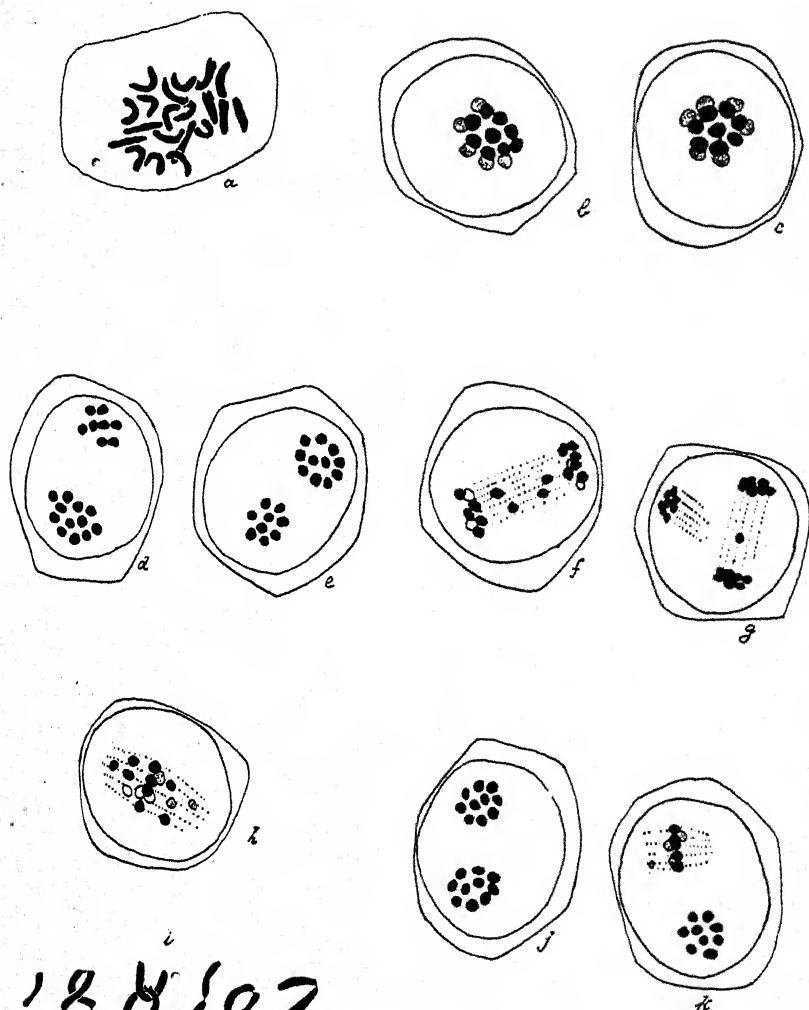
Text-fig. 6. *a.* Somatic metaphase in the root tip of the hypotriploid *Petunia* with 20 somatic chromosomes; *b–k*, reduction division in the hypotriploid *Petunia*: *b*, *c*, heterotypic metaphases; *d*, *e*, *j*, *k*, homeotypic metaphases with 8, 9, 10, 11, and 12 chromosomes; *f*, *h*, heterotypic anaphases; *g*, homeotypic anaphase; *i*, single chromosomes and groups of twos and threes during the diakinesis.

Sections of the root tips of this plant showed 20 chromosomes present in the somatic metaphases (Text-fig. 6 *a*). Its cytological behaviour as observed in the flower buds during meiosis resembles very much that of

the triploid plant just described. In diakinesis the chromosomes were arranged diversely, some of them appeared singly, others associated variously in twos, and still others appeared linked in a group of three (Text-fig. 6 *i*). In the late heterotypic metaphase 14 chromosomes appeared; some of these tended to join in the earlier heterotypic metaphase as shown in Text-fig. 6 *b*, *c*, so that 8, 9, 10, or 11 chromosomes were often counted. This reminds one of the behaviour of the chromosomes during this phase in the triploid. The heterotypic division was observed to be much more irregular than that in the triploid, chromosomes leading the advance to the poles (Text-fig. 6 *h*) and lagging on the spindle (Text-fig. 6 *f*) were more numerous and occurred more frequently. The chromosome distribution to the poles during the heterotypic division varied, so that during the homeotypic metaphases the two plates were observed with the following sets of chromosomes: 7 and 13, 8 and 12, 9 and 11, and 10 and 10. Most frequently, however, 10 : 10 (Text-fig. 6 *j*), 9 : 11 (Text-fig. 6 *e*), and 8 : 12 (Text-fig. 6 *d*) were counted. The homeotypic division was more regular than the heterotypic one, but more irregular than in the case of the triploid plant, single chromosomes leading the advance to the poles or lagging on the spindle (Text-fig. 6 *g*) were observed very often.

As the result of such irregularities in the meiotic process the pollen of this hypotriploid plant was found to contain a very high percentage, about 50–55 per cent., of abortive grains. All attempts to self it were unsuccessful, and it set only relatively very few seeds when pollinated by pollen from the tetraploids.

The hypotriploid plant apparently originated from a fusion of a 13-chromosome egg nucleus of the maternal tetraploid plant and a normal 7-chromosome paternal gamete. A fusion of a 14-chromosome egg nucleus with a 6-chromosome gamete from the father seems very improbable, since the haploid number is 7 and all gametes with less than 7 appeared to be non-viable, while a 13-chromosome maternal gamete has a complete haploid chromosome set and an additional 6 chromosomes from another set.

On the basis of the study of the 20 plants of the progeny, 3 of which (2 hypotetraploids and 1 hypotriploid) presumably originated from a maternal gamete with 13 chromosomes, the ratio of formation of 13- and 14-chromosome gametes in the mother tetraploid plant was about 1 : 6.

SUMMARY.

After crossing a tetraploid *P. violacea* (28 somatic chromosomes) with a diploid (14 somatic chromosomes) which differed in certain characters from the tetraploid, progeny were obtained with 28 (tetraploid), 27 (hypotetraploid), 21 (triploid), and 20 (hypotriploid) chromosomes, and diverse morphological characters.

The appearance of the plants with one less chromosome than the complete tetraploid or triploid set is attributed to the irregular meiotic divisions in the tetraploid mother plant. The ratio of formation of 13- and 14-chromosome gametes in this plant as a result of non-disjunctions in the reduction division was calculated to have been 1 : 6.

Flower buds of the progeny studied during meiosis showed meiosis to be accompanied by various degrees of irregularities. This process was almost regular in the tetraploids, only slightly less so in the hypotetraploids, quite irregular in the triploid, and most irregular in the hypotriploid.

The plants with 28 and 27 somatic chromosomes were self-fertile, those with 21 and 20 were self-sterile but set seed when pollinated with the tetraploids, and in the case of the triploid with the hypotetraploids also.

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DESCRIPTION OF PLATE III.

Fig. 1. The hypotriploid plant with 20 somatic chromosomes.
Fig. 2. Hypotetraploid plant no. 16 with 27 somatic chromosomes.
Fig. 3. A tetraploid plant with 28 somatic chromosomes.
Fig. 4. The triploid plant with 21 somatic chromosomes.
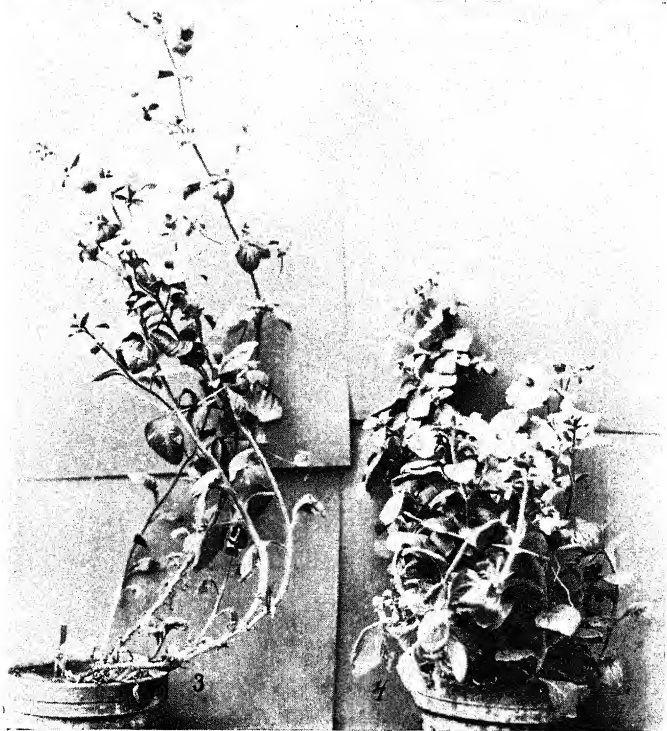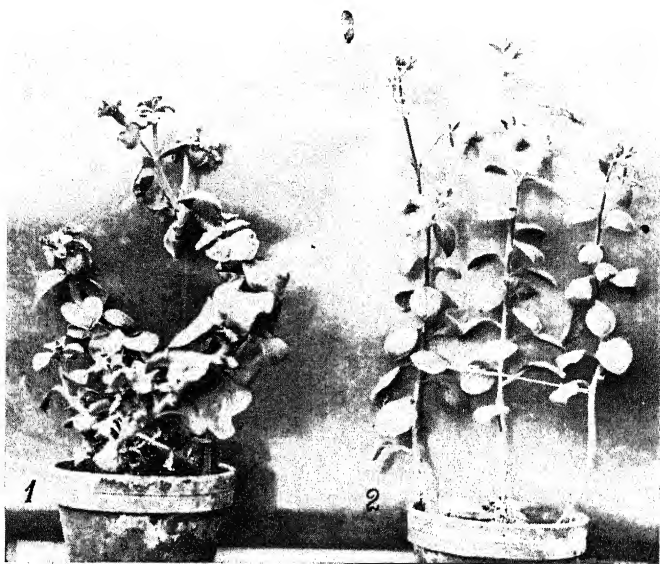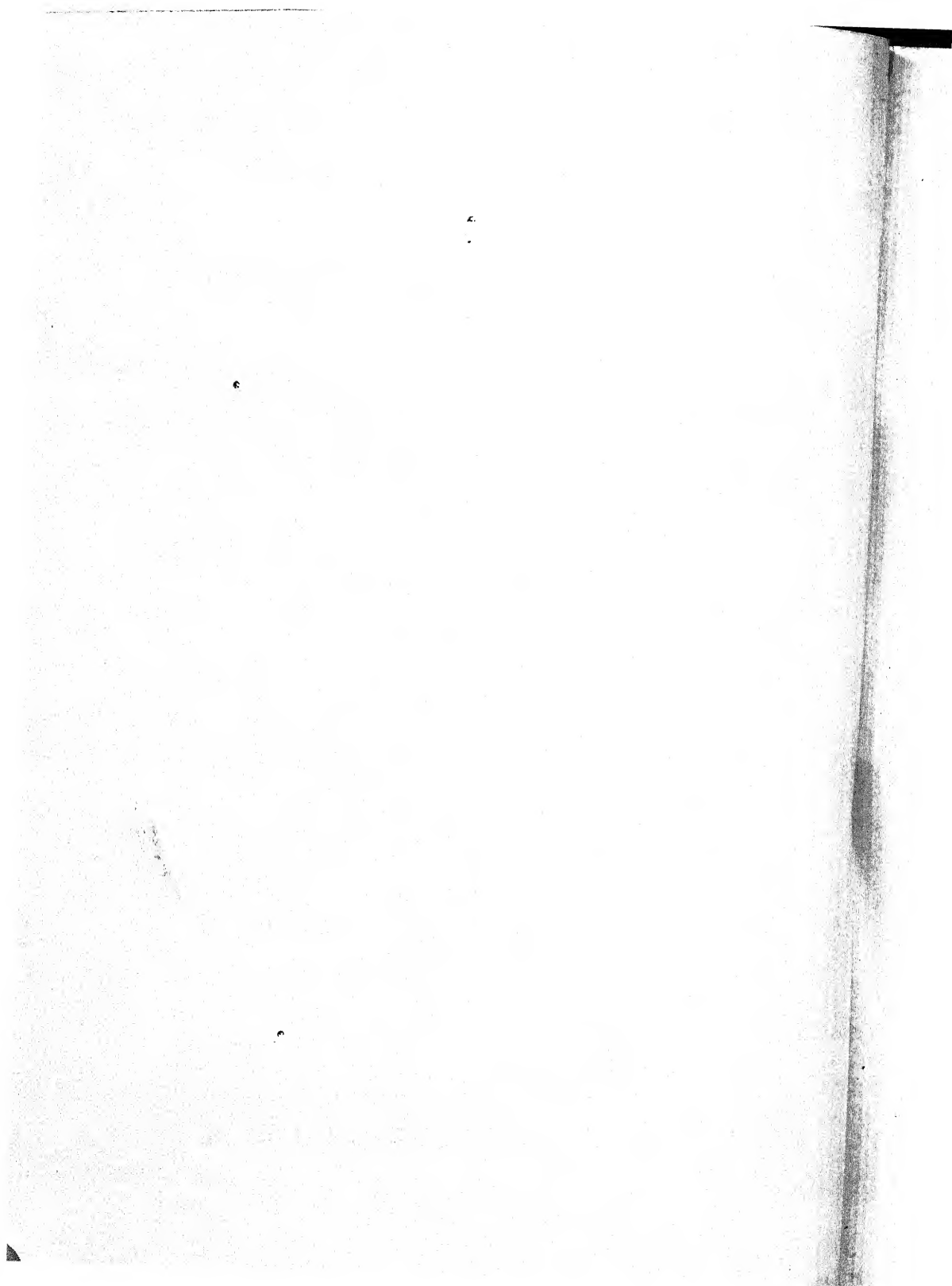
BEHAVIOUR OF TWO MUTABLE GENES OF *DELPHINIUM AJACIS*[1].

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(With Plate IV and Seven Text-figures.)

INTRODUCTION.

THE name "mutable genes" is applied to genes which are in an unstable condition, mutating with a relatively high frequency. In all of the mutable genes which we had studied, both in *Delphinium* and in *Drosophila*, the mutability was of a reversion type, viz. the mutant gene reverted frequently to its wild-type allelomorph. These reversions, occurring in somatic cells, produce mosaic individuals having two kinds of tissue. When dealing with mutable plant colour characters, the mosaic appearance produced by somatic reversions is called variegation. It is evident that from a mutation in somatic cells which occurs during the early stages of somatic development, a large region of changed tissue is produced, and from a mutation which occurs late in the somatic development, a small region of changed tissue results. The size of the spots of changed tissue, therefore, can be used as an indication of the time when the mutation occurred, large spots being the early mutations and small spots being the late ones.

It is intended to present here a short summary of the data on the behaviour of mutable rose-alpha and lavender-alpha genes of *Delphinium*. An extensive account of this work is being prepared for another publication.

ROSE-ALPHA GENE.

The gene for the rose-alpha flower colour mutates frequently to its purple wild-type allelomorph. From the seed of a self-pollinated rose-alpha variegated plant, in addition to variegated plants, a few purple and a few rose-variegated and purple chimeral plants are obtained. Purple plants originate through reversions from rose-alpha to purple, which affect the germ cells. The chimeral plants with large purple and

rose-variegated sectors are the result of somatic reversions which occurred very early in the development of the plant. Both the solid purple and the chimeral plants are rare.

The mutations occurring in the epidermal cells of sepals and petals show up as purple spots (Text-fig. 1). When the purple spots are examined
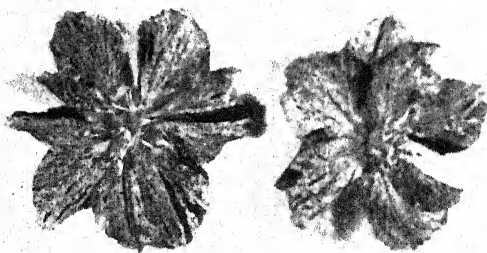
Text-fig. 1. Flowers showing varietations produced by frequent mutability of the rose-alpha gene.
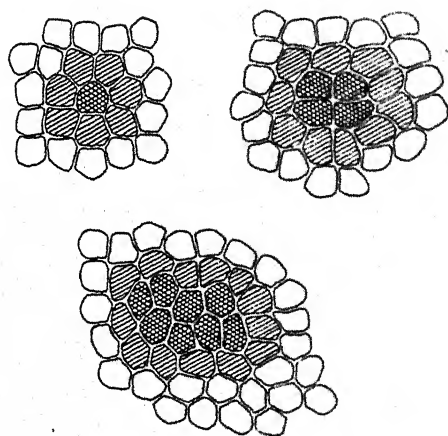
Text-fig. 2. Purple spots produced by reversions of rose-alpha gene. Dark purple cells are darkly shaded, light purple cells are lightly shaded, and rose cells are unshaded.

under a magnification of 25 to 30 times, it can be noticed that the smallest spot has one dark purple cell in the centre, surrounded by a layer of distinctly lighter purple cells (Text-fig. 2). In the larger spots, also, uniformly purple cells in the centre are always surrounded by a layer of distinctly lighter purple cells. It is assumed that the dark purple cells only have the reverted purple allelomorph of rose-alpha, and that the light purple colour of the cells on the borderline of purple spots is produced

by some substance which diffuses from the dark purple cells into the adjacent pink cells. A study of the cell lineage supports that assumption. The smallest purple spot, therefore, has one reverted cell only, and, since it is possible to count the number of dark purple cells in the large spots, the cell can be used as a unit in measuring the size of different purple spots.

It has been mentioned already that the size of the spots can be used as an indication of the cell generation in which the mutation occurred. It may be assumed that, on the average, a mutation responsible for a spot having twice as many cells as another spot has occurred one cell generation earlier, at which time the total number of cells was only one-half as great. It is evident that, due to irregularities in cell division, the relationship between the number of cells and the cell generation is not a perfect one. When dealing with large numbers, however, an analysis based on the above assumption gives us probably a close enough approximation to the actual facts to warrant its use.

The method used in collecting the data was as follows. Sepals only were used in the observations. The area of each sepal was measured. Since the measurements indicated that the size of cells on sepals is very constant and that about 900 cells cover an area of 1 sq. mm., by knowing the area of the sepals the total number of cells could be computed. The purple spots present on the sepals were counted and classified, according to the number of purple cells they contained, into classes varying from 1 to 4096 cells, each class having double the number of cells of the preceding one. Each class theoretically represented mutations which occurred during the same cell generation. The spots with one purple cell are the mutations which occurred in the last cell generation of the development of the sepals (the twelfth cell generation in Text-figs. 3 to 5). Since the total number of cells in the last cell generation could be determined from the area of the sepals, the number of cells of the previous generations could be computed also, and the rate of mutations per certain number of cells could be calculated for each cell generation.

In Table I a summary of the data is given, which was used to compute the rate of mutability of rose-alpha gene for the last twelve cell generations in the development of sepals. These data are graphically shown in Text-fig. 3, from which it can be seen that the mutability curve for rose-alpha gene for twelve successive cell generations is practically a horizontal line.

Flowers on a *Delphinium* plant are arranged in a spiral. In general, two twists of the spiral, involving a set of five flowers, complete one cycle,

bringing the sixth flower into a position directly over the first, the seventh over the second, etc., for the next set of five. Two consecutive sets of five flowers of the same raceme, therefore, are comparable as far as the position of the flowers is concerned, and are several cell generations apart in the development. In Text-fig. 4 curves are given for the rate of mutability of rose-alpha gene in a set of the first five flowers and in a

TABLE I.

Data showing the frequency of purple spots grouped in classes according to number of cells they contained.

Nos. measured			Total area of sepals (mm.²)
Plants	Flowers	Sepals	
80	642	3213	187,480

Frequency of purple spots classified according to size in terms of cell numbers

1	2	4	8	16	32	64	128	256	512	1024	2048	4096
16352	5558	3590	1438	802	332	178	92	47	22	14	5	1
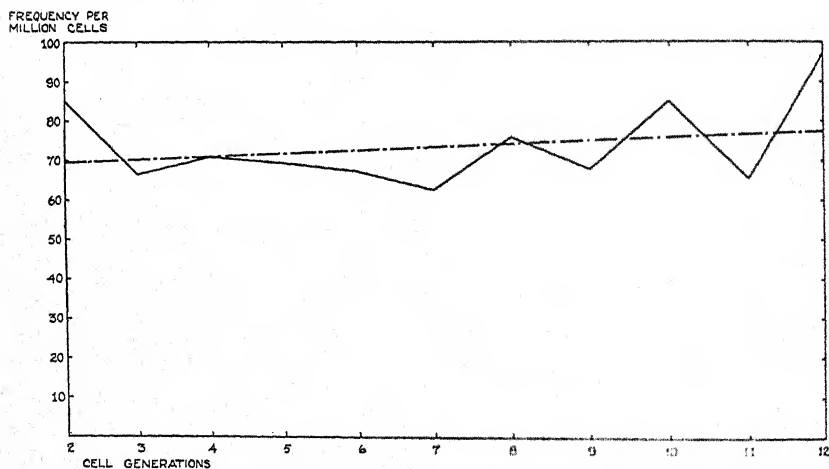
Text-fig. 3. Rate of mutability of rose-alpha gene for the last twelve cell generations in the development of sepals.

set of the second five flowers of the same racemes. It is evident that the rate of mutability is practically identical in the lower set of flowers and in the higher set of flowers. Text-fig. 4 indicates, also, that the curves for the rate of mutability of rose-alpha gene during the development of sepals are horizontal lines.

The mutability curve of Text-fig. 3 is based on data collected from plants belonging to five families. Text-fig. 5 gives the mutability curves

of each of these families separately. All of these curves are practically horizontal lines, not differing greatly from the curve of Text-fig. 3.

The material used in measurements of the rate of mutability of rose-alpha gene, segregated for a Mendelian factor which greatly reduced the rate of mutability. Consequently, in some of the plants the rose-alpha gene mutated with a very low frequency, and in others the frequency of
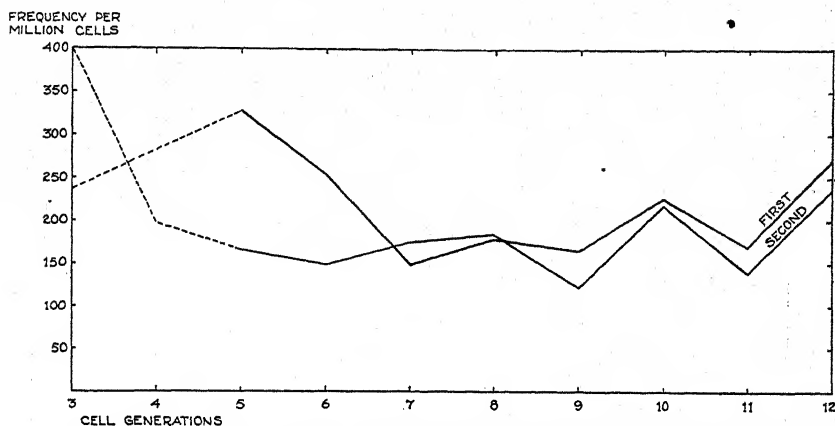
Text-fig. 4. Comparison of the rate of mutability in first five flowers with the rate of mutability in second five flowers of the same raceme.
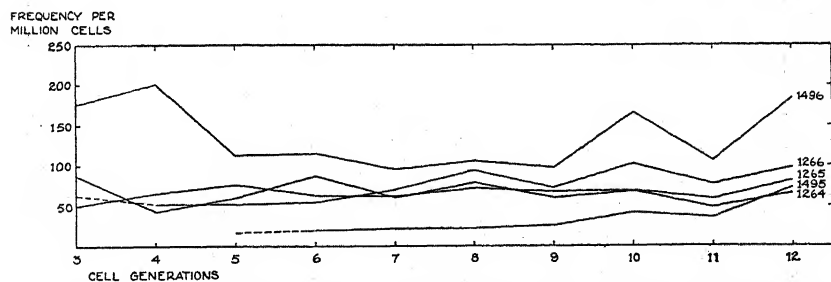
Text-fig. 5. Rate of mutability of rose-alpha gene in five different families.

mutability was quite high. Plants were placed into four groups according to the frequency with which rose-alpha gene mutated. The curves for the rate of mutability of rose-alpha gene in these four groups are given in Text-fig. 6. The curves are practically horizontal lines, indicating that the rate of mutability was practically constant for ten cell generations of the development of the sepals in the material which had a low frequency of mutability, as well as in the material which had a high frequency of mutability.

Solid purple plants appearing among the offspring of variegated parents indicate mutations occurring during the gametogenesis. Since any mutation which occurred during the early stages of gametogenesis produces several changed germ-cells, the number of purple plants is expected to be greater than the rate of mutability, but the mutation rate can be estimated from the total number of purple plants. If the rate of mutability during the gametogenesis is constant, as we are justified in
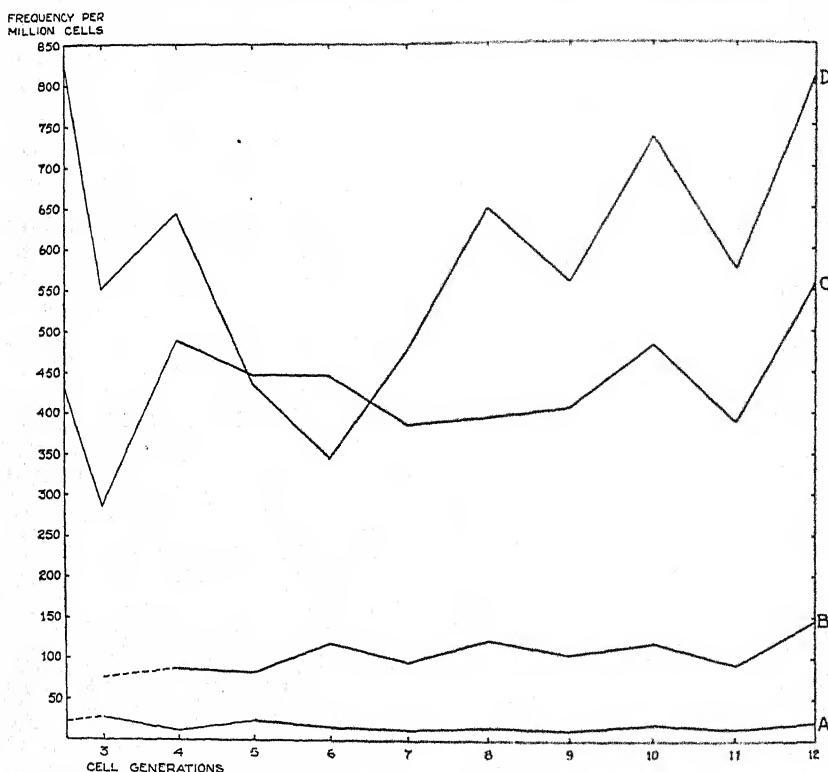
Text-fig. 6. Mutability curves for rose-alpha genes mutating with different frequencies.

assuming, having found it constant during the development of the sepals, then the rate of mutability of any cell generation is approximately one-third of the total number of purple plants. Mutations which might have occurred in somatic tissue prior to gametogenesis would have distorted these data but, had such mutations occurred, they would have been recognised by a large proportion of purple plants in their offspring.

To determine the rate of mutability for rose-alpha gene in gameto-genesis, several variegated plants, which had a fairly uniform rate of

mutability (the average being about 350 mutations per million cells), were tested. Among the offspring of these plants 875 were variegated and 8 were purple. That would indicate a rate of mutability of 267 per million cells in the gametogenesis of these plants, a value close to that of the mutability in the sepals.

The mutability of rose-alpha gene has been studied for four sexual generations in the offspring of a single self-pollinated plant. That plant was heterozygous for rose-alpha and lilac flower colour and, therefore, all of the rose-alpha genes of the subsequent generations traced their origin to a single gene. Since lilac is an allelomorph of rose-alpha, it was possible to distinguish the homozygous from the heterozygous individuals in the offspring of the self-pollinated plants, because the homozygous rose-alpha plants are rose with purple variegations and heterozygous rose-alpha individuals are lilac with purple variegations. The line was propagated by selfing lilac variegated plants. The data from measurements made on heterozygous rose-alpha plants only were used in this study; and, therefore, the mutability of a single rose-alpha gene is compared during four sexual generations. Table II gives a summary of the data.

TABLE II.

Average rate of mutability of rose-alpha gene for four generations of inbreeding.
(The rate of mutability is expressed in terms of numbers of mutations per 1000 sq. mm.)

1st generation	2nd generation	3rd generation	4th generation
			(a) 1588 ± 112.1, σ551 ± 79.3
			(b) 1413 ± 62.7, σ336 ± 44.4
1182 ± 90.3, σ378 ± 63.8	1198 ± 66.9, σ298 ± 47.3	(a) 1241 ± 109.2, σ606 ± 77.2	(c) 1512 ± 71.6, σ411 ± 52.0
			(d) 1581 ± 86.6, σ445 ± 61.2
		(b) 1267 ± 47.9, σ309 ± 33.9	(e) 860 ± 35.0, σ156 ± 24.8
			(f) 1239 ± 94.4, σ464 ± 66.8

The rate of mutability in that table is expressed in terms of numbers of mutations (purple spots) per 1000 sq. mm. of the sepals. Each value is an average mutability rate for 8 to 19 heterozygous plants of a family. It is evident from Table II that out of ten families tested the (e) family of the fourth generation is the only one which had a significantly different rate of mutability. The rate of mutability of rose-alpha gene was approximately the same in all of the other nine families. The (e) family of the fourth generation is being tested to determine whether the lower rate

was due to a change in the rose-alpha gene or was caused by some other factor.

As can be seen from the standard deviations, the variability in the rate of mutability was high among the plants of the same family. That this variability was due to a large extent to non-hereditary causes is indicated by the fact that the offspring of the plants having a low rate of mutability did not differ from the offspring of the plants having a high rate of mutability. The parent plant of family (*d*) of the fourth generation, for example, had a rate of mutability of 647 and the parent plant of the family (*f*) had a rate of mutability of 2803, but the average rate of mutability of the offspring of these two families was not significantly different.

In summarising the behaviour of rose-alpha gene it may be stated that it is in an unstable condition reverting (mutating) frequently to its purple wild-type allelomorph. The results of the experiments indicate that the rate of mutability is to a high degree a regular process. The evidence indicates (1) that the rose-alpha gene mutated with the same rate during twelve cell generations of the development of the sepals; (2) that the rate of mutability was the same when the mutability of the gene in the lowest five flowers was compared with the mutability in the next five flowers of the same racemes; (3) that the curves for the rate of mutability during several cell generations of the development of sepals were practically horizontal lines in material differing widely in frequency of mutability; and (4) that the rate of mutability of the gene during gametogenesis did not differ greatly from the rate of mutability in the petals. The experimental evidence, furthermore, indicates a high degree of stability in the rate of mutability of rose-alpha gene during four sexual generations. Out of ten families tested only one differed significantly in the rate of mutability. If this different rate of mutability proves to be due to a change in the rose-alpha gene, that change would be comparable to changes occurring between the alpha, beta and gamma allelomorphs of the mutable miniature gene of *Drosophila virilis* (Demerec, 1929 *b*).

LAVENDER-ALPHA GENE.

The lavender-alpha gene mutates frequently to its purple allelomorph. Lavender-variegated, purple, and purple-lavender chimeral plants are obtained from the seed of self-pollinated lavender-variegated plants.

A close examination of the lavender-variegated flowers reveals that they have small purple spots only (Plate IV, fig. 1). Purple spots of intermediate or large size are missing or, if present, the origin of the

majority of them can be traced to a fusion of several small spots. It is unfortunate that the purple colour of the spots diffuses through several layers of cells gradually becoming lighter. This makes it impossible to determine exactly how many purple cells are involved in each of the spots. However, numerous small purple spots and a small number or an entire absence of intermediate and large spots indicates that the lavender-alpha gene has a high rate of mutability towards the end of the development of the sepals and petals, and it has a low mutability rate, or it does not mutate at all, in the earlier stages of that development.

Several selfed light variegated lavender-alpha plants gave the offspring as follows: 92 lavender-variegated plants; 89 lavender-purple chimeras with purple sectors extending over more than one-fifth of the plants (Plate IV, fig. 2); and 169 purple plants.

Among the non-purple plants, therefore, about 50 per cent. were chimeras indicating that the rate of mutability of lavender-alpha gene was very high in the early stages of the development of these plants. A large number of solid purple plants among the offspring of the lavender-variegated parents might indicate a high rate of mutability during the gametogenesis, or it might be a result of changes which occurred prior to the gametogenesis.

The type of mutability of the lavender-alpha gene, therefore, is strikingly different from the type of mutability of the rose-alpha gene. As already pointed out earlier, rose-alpha gene has a constant rate of mutability during the development of the sepals, has approximately the same mutability rate during the gametogenesis, and, since chimeral plants with large purple sectors are very rare, the rate of mutability of the rose-alpha gene during the early development of the plant is at any rate not higher than the mutability rate in the sepals. The lavender-alpha gene, on the other hand, has a high rate of mutability in the early stages of the development of the plant, a low mutability rate, or is stable during the early stages of the development of the sepals and petals, and again a high rate of mutability towards the end of the development of the sepals and petals.

DISCUSSION.

The evidence available at present on the behaviour of mutable genes suggests that the frequent mutability of these genes is not an irregular process, but that it is a well-regulated activity of the genes. Of the two mutable genes of *Delphinium ajacis*, rose-alpha gene mutated with the same rate throughout all stages of the ontogeny for which the measure-

ments were obtained; also, the gene retained to a high degree the same rate of mutability throughout four sexual generations. Lavender-alpha gene, on the contrary, had a variable rate of mutability during the life cycle of the plant, the rate being high early in the development of the plant and late in the development of the sepals and petals, and low during the early development of sepals and petals. Of the three mutable genes known in *Drosophila virilis* (Demerec, 1926, 1927, 1928) reddish-alpha mutates at the reduction division of heterozygous females only, magenta-alpha and miniature-alpha mutate in the germ cells and in the somatic tissue; miniature-gamma mutates in the somatic tissue only, and miniature-beta does not revert to the wild type at all, but changes with a low frequency to the more mutable gamma or alpha forms. It appears as if a mutable gene becomes unstable whenever the cell reaches a certain stage in the development of the organism where, probably, the chemical properties of the cytoplasm are such that they stimulate the reversions in the genes. Different genes, apparently, require different conditions to become mutable. For the reddish-alpha gene, for example, that requirement is fulfilled at the reduction division of heterozygous females, for the lavender-alpha gene early and late in the development of the plant and for the rose-alpha gene during all stages of the development of the plant. The evidence obtained in experiments with miniature-gamma (Demerec, 1929 *a*, *b*) and with rose-alpha indicates that under a given set of conditions the rate of mutability for certain genes is constant.

It has been shown for mutable miniature of *Drosophila virilis* (Demerec, 1929 *b*) that the gene changes in two directions, viz. it reverts to the wild-type allelomorph and also changes into mutable allelomorphs having different rates of mutability (Text-fig. 7). The data, which are at present available, indicate a similar behaviour for the mutable rose gene of *Delphinium*. In the case of both of these mutable genes, however, changes to the allelomorphs of a different mutability rate are less frequent than changes to the wild-type allelomorphs.

To explain the frequent mutability of mutable genes it has been suggested (E. G. Anderson, 1921, not published; Eyster, 1924) that these genes are complex structures composed of two kinds of smaller genetic units, and that the frequent changes in mutable genes are produced due to the elimination of one kind of these units by a mechanical sorting-out process. The evidence obtained in experiments with rose-alpha gene contradicts the above hypothesis. It is mathematically impossible to conceive a sorting-out process acting during the ontogeny of a plant which would produce a constant rate of mutability of about seventy per

million during twelve successive cell generations. The difficulty is still greater when an attempt is made to explain the same rate of mutability found in flowers located low on the racemes and flowers located high on the same racemes, and also when an explanation is sought for practically constant mutability curves in plants differing widely in the rate of mutability. Another strong point against the complex-gene hypothesis is the unchanged rate of mutability observed during the four generations of sexual reproduction. According to that hypothesis genes having different proportions of particles and, therefore, different rates of mutability should have been obtained after sexual reproduction. In brief, the results of the experiments with rose-alpha gene render untenable the hypothesis that the gene is a physically complex structure composed of smaller independent hereditary units, and that the frequent mutability
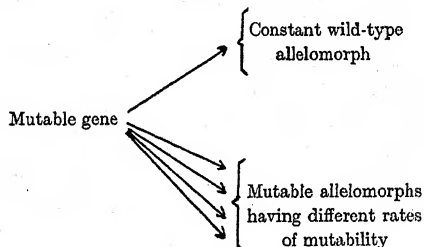
Text-fig. 7. A diagram showing the type of changes observed in mutable genes.

of mutable genes is a result of an assortment of these particles. It appears probable that the cause of frequent mutability of mutable genes is a high chemical lability of these genes, and that the changes occurring in mutable genes are due to chemical changes rather than to a physical assortment of gene particles.

The constant rate of mutability of the rose-alpha gene during twelve generations of the development of sepals indicates that the gene mutated with an equal frequency per cell generation rather than per unit of time, since the time element was undoubtedly different for different cell generations. That was especially true for the last cell generation which lasted from the end of the last cell division until the death of the tissue. The mutability rate, therefore, would not have been constant if the time element influenced the mutability. The constant rate of mutability per cell generation suggests that the gene does not mutate throughout all stages of cell division; but that the mutability is limited to a certain period of that division, and that this period is of approximately equal duration in different cell generations. This assumption would eliminate

TABLE III.

Data for curves of Figs. 3, 4, 5 and 6.

Cell generations*

Fig. 3

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	82,389	164,778	329,555	659,110	1,318,219	2,636,438	5,272,875	10,545,750	21,091,500	42,183,000	84,366,000	168,732,000
...ons	5	14	22	47	92	178	332	802	1438	3590	5558	16352
per 1,000,000 cells	60·7	85·0	66·8	71·3	69·8	67·5	63·0	76·1	68·2	85·1	65·9	96·9

Fig. 4

First five flowers

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	4,181	8,363	16,727	33,454	66,909	133,818	267,637	535,275	1,070,550	2,141,100	4,282,200
...ons	—	1	2	1	11	17	20	48	88	243	362	1155
per 1,000,000 cells	—	—	—	—	328·8	254·1	149·5	179·4	164·4	227·0	169·1	269·7

Second five flowers

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	—	7,502	15,004	30,009	60,018	120,037	240,075	480,150	960,300	1,920,600	3,841,200
...ons	—	—	3	3	5	9	21	44	59	210	268	903
per 1,000,000 cells	—	—	399·9	200·0	166·6	150·0	175·0	183·3	122·9	218·7	139·5	235·1

Fig. 5

Family 1264

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	—	45,826	91,652	183,305	366,610	733,219	1,466,438	2,932,875	5,865,750	11,731,500	23,463,000
...ons	—	—	4	4	11	32	44	116	177	407	585	1571
per 1,000,000 cells	—	—	87·3	43·6	60·0	87·3	60·0	79·1	60·4	69·4	49·9	67·0

Family 1265

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	79,128	158,256	316,512	633,024	1,266,047	2,532,094	5,064,188	10,128,375	20,256,750	40,513,500	81,027,000
...ons	—	6	8	21	49	79	156	367	696	1417	2418	6594
per 1,000,000 cells	—	75·8	50·6	66·4	77·4	62·4	61·6	72·5	68·7	70·0	59·7	81·4

Family 1266

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	11,958	23,915	47,830	95,660	191,320	382,640	765,281	1,530,563	3,061,125	6,122,250	12,249,500	24,489,000
...ons	1	2	3	5	10	21	54	144	222	626	946	2415
per 1,000,000 cells	60·7	83·6	62·7	52·3	52·3	54·9	70·6	94·1	72·5	102·3	77·3	98·6

* Twelfth cell generation is equivalent to one-cell spots, eleventh to two-cell spots, tenth to four-cell spots, etc.

TABLE III (*continued*).

Cell generations*

Fig. 6

Family 1495

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	27,772	55,543	111,086	222,172	444,344	888,688	1,777,375	3,554,750	7,109,500	14,219,000	28,458,000
...ons	—	1	—	1	4	9	19	40	94	298	519	2058
per 1,000,000 cells	—	—	—	—	18	20·3	21·4	22·5	26·4	41·9	36·5	72·3

Family 1496

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	9,910	19,819	39,639	79,277	158,554	317,109	634,219	1,268,438	2,536,875	5,073,750	10,147,500	20,295,000
...ons	3	4	7	16	18	37	59	135	249	842	1090	3714
per 1,000,000 cells	—	201·8	176·6	201·8	113·5	116·7	93·0	106·4	98·2	166·0	107·4	183·0

Group A

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	122,379	244,758	489,516	979,031	1,958,062	3,916,125	7,832,250	15,664,500	31,329,000	62,658,000	125,316,000
...ons	—	2	7	6	23	32	56	136	219	631	885	2710
per 1,000,000 cells	—	16·3	28·6	12·3	23·5	16·3	14·3	17·4	14·0	20·1	14·1	21·6

Group B

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	—	28,283	56,566	113,133	226,266	452,531	905,062	1,810,125	3,620,250	7,240,500	14,481,000	28,962,000
...ons	—	1	4	10	19	54	86	221	378	866	1342	4207
per 1,000,000 cells	—	—	70·7	88·4	84·0	119·3	95·0	122·1	104·4	119·6	92·7	145·3

Group C

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	4,337	8,675	17,349	34,699	69,398	138,797	277,594	555,188	1,110,375	2,220,750	4,441,500	8,883,000
...ons	1	5	5	17	31	62	107	219	451	1072	1725	4932
per 1,000,000 cells	—	576·4	288·2	489·9	446·7	446·7	385·5	394·7	406·2	482·7	388·4	555·2

Group D

	1	2	3	4	5	6	7	8	9	10	11	12
...ons	2,720	5,440	10,881	21,762	43,523	87,047	174,094	348,187	696,375	1,392,750	2,785,500	5,571,000
...ons	3	6	6	14	19	30	83	226	390	1021	1606	4503
per 1,000,000 cells	—	1102·9	551·4	643·3	436·6	344·6	476·8	649·1	560·0	733·1	576·6	808·3

* Twelfth cell generation is equivalent to one-cell spots, eleventh to two-cell spots, tenth to four-cell spots, etc.

the resting period of the nucleus as a stage during which mutations might occur, since the duration of the resting periods in the various cell generations is different. The early stages of cell division, when the chromosomes are not as yet split, could be eliminated also, since, if the mutations were to occur at those stages, they would produce two changed cells and would increase the frequency of two-cell spots, or, if mutations were limited to that stage of cell division, two-cell spots would be expected tó be the smallest spots found. Since the two-cell spots are not relatively more frequent than the one-cell spots, it may be assumed that the gene is not mutable at the early stages of cell division. The mutability period of rose-alpha gene, therefore, is probably limited to the stage of cell division which begins with the gene division and ends before the nucleus reaches the resting stage. During that period of cell division the most significant change, as far as the gene is concerned, occurs when two genes are formed from one, and it appears probable that this might be the period when the gene is in an unstable condition.

If we assume that rose-alpha gene is unstable at the time of gene divisions only, a speculation could be made as to the nature of gene divisions. The gene might divide in one of two possible ways: (1) it might split into two genes, or (2) the new gene might be formed next to the old gene from the material accumulated from the cytoplasm by the action of the old gene. If the gene multiplies by the division of the old one, and that division produces two equal parts then, whenever a change in the rose-alpha gene occurs, it could be expected to affect at least two cells. If the new gene is formed independently from the old one, a change in the gene would affect one cell only. The first assumption would make the occurrence of one-celled purple spots in the case of mutable rose-alpha impossible, and since they do occur the experimental evidence makes the second assumption more propable. An unequal division of a gene might also be responsible for the occurrence of a gene mutation. It might be expected, however, that unequal divisions would produce quantitatively different genes which, when dealing with mutable genes, would differ in their rate of mutability. In the case of rose-alpha gene just the opposite has been observed.

SUMMARY.

Rose-alpha gene mutates frequently to its purple allelomorph. The results of measurements indicate that the gene mutated with approximately the same rate during twelve cell generations of the development of sepals. No difference in mutability was observed when the gene
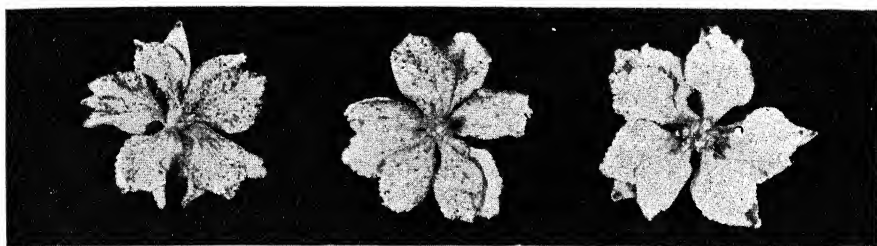
Fig. 1.
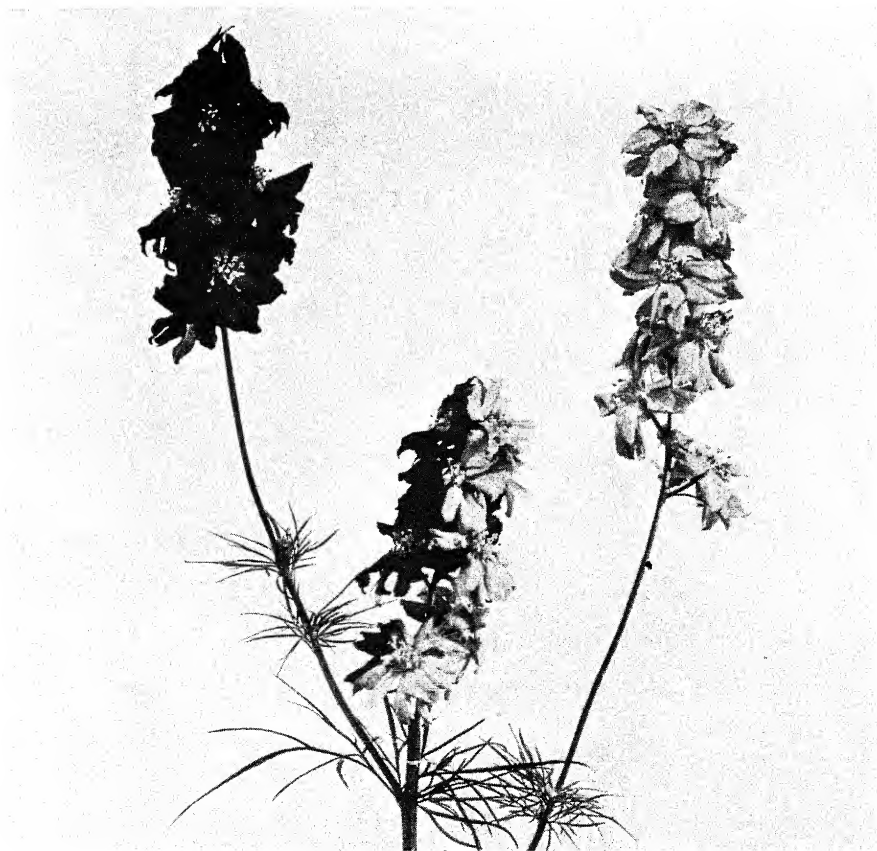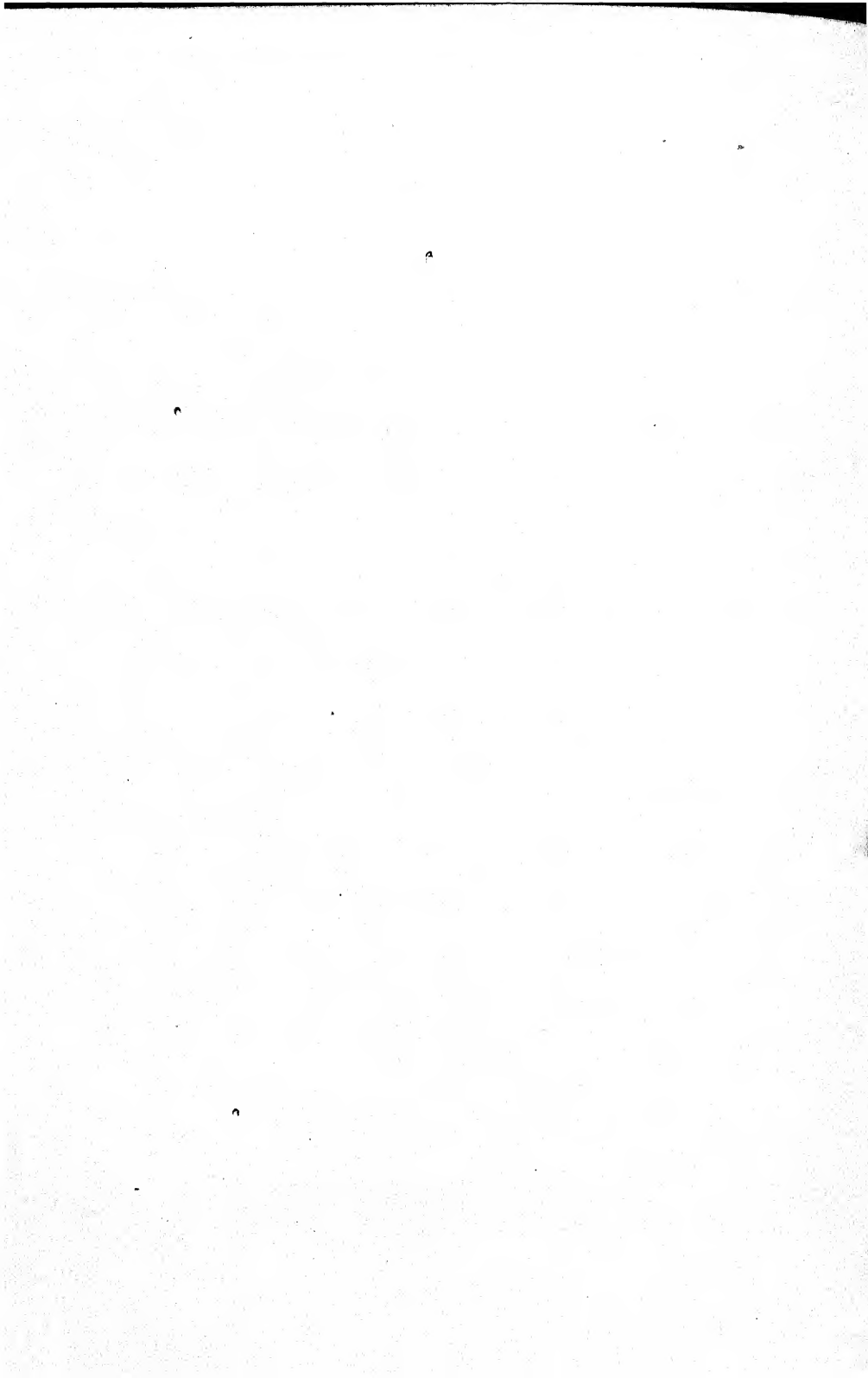
mutability in the lowest five flowers of racemes was compared with the mutability of the next five flowers of the same racemes. The mutability curves were practically horizontal lines in material differing widely in the frequency of mutability. The rate of mutability in gametogenesis was probably not higher than the rate of mutability in somatic tissue.

Lavender-alpha gene has a high rate of mutability early and late in ontogeny, and has a low rate of mutability or is constant in the intermediate stages of ontogeny.

The evidence accumulated in studying mutable genes is opposed to the assumption that these genes are composed of two kinds of smaller hereditary units (gene elements), and that the frequent mutability of these genes is due to an assortment of gene elements.

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EXPLANATION OF PLATE IV.

Fig. 1 Flowers showing variegations produced by frequent mutability of the lavender-alpha gene.

Fig. 2. A lavender-purple chimera plant showing large sectors of lavender-variegated and of purple tissue.
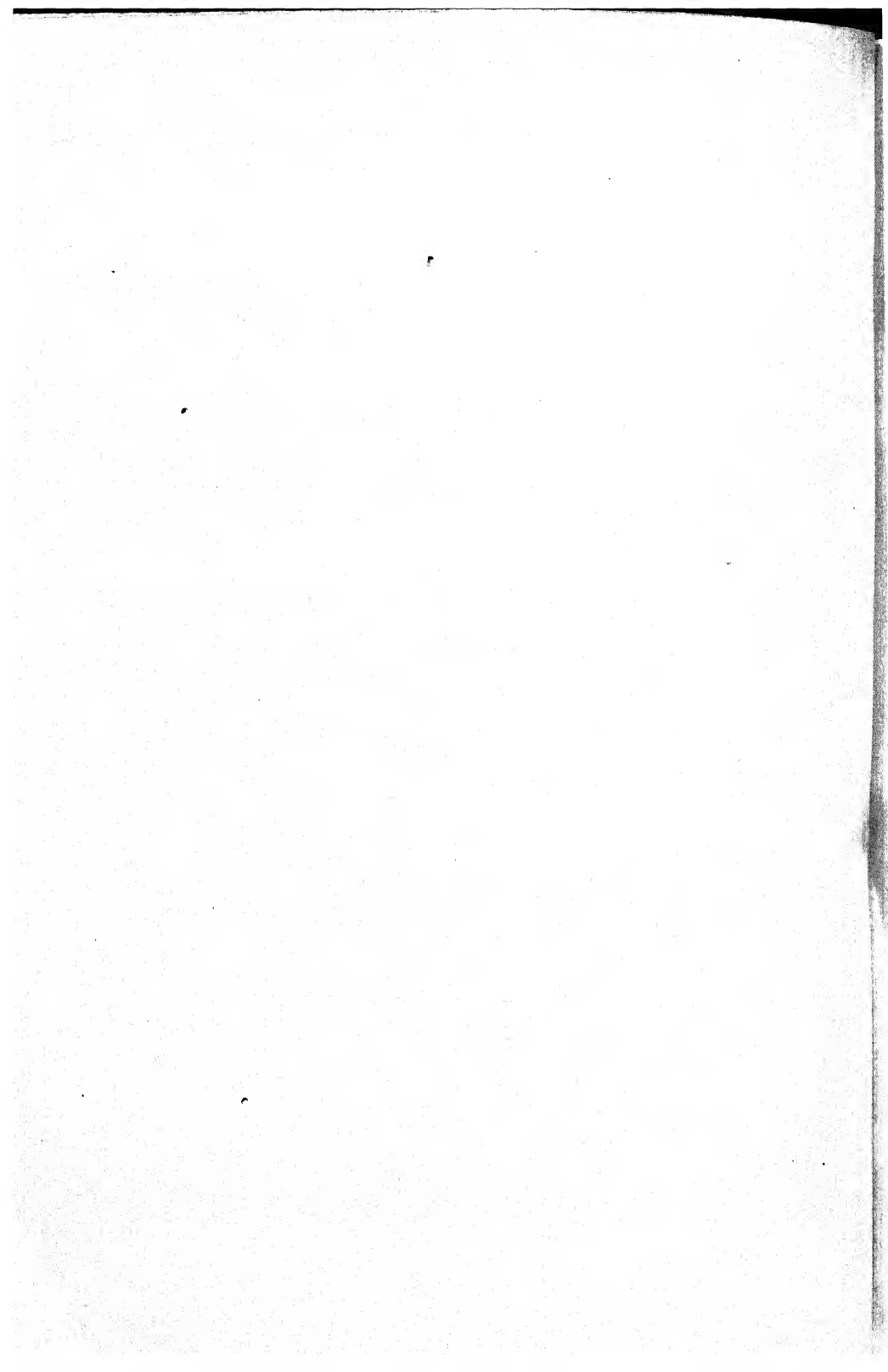
FURTHER NOTES ON THE TORTRICID MOTH
ACALLA COMARIANA ZELLER.

By J. C. F. FRYER, M.A.

A PRELIMINARY account of the inheritance of wing coloration and pattern in the polymorphic species, *A. comariana*, appeared in a previous issue of this *Journal**. Experimental work had then been undertaken with six different forms, *e.g. proteana, potentillana, latifasciana, fasciana, brunneana* and *comparana*, and the existence of a seventh form, *fuscana*, noted. For a full description and coloured pictures of these seven forms, the reader is referred to the original paper, but for convenience the essential distinctions between these forms may be briefly outlined as follows:

Forewings grey	{ with brown costal blotch	*proteana* Hg.
	{ with black ,, ,,	*potentillana* Cooke
Forewings marbled brown	{ with brown ,, ,,	*latifasciana* Sheldon
and white	{ with black ,, ,,	*fasciana* Sheldon
Forewings brown	{ with brown ,, ,,	*brunneana* Sheldon
	{ with black ,, ,,	*comparana* Sheldon
Forewings dark purplish fuscous or fuscous-black; costal blotch hardly differentiated by colour		*fuscana* Sheldon

As a result of the experiments, it was suggested that inheritance of the first six forms mentioned above could be explained by assuming (1) that the ground colours of the wing are due to a series of three multiple allelomorphs **B** (brown), **B**¹ (marbled), **B**² (grey); (2) that the black costal blotch is due to a factor **C**, in the absence of which (c) the blotch is brown; and (3) that there is a close linkage between the costal blotch colour and ground colour, all the factors being in a pair of autosomes. This explanation has proved adequate in the experiments subsequently carried out, but it may be well to give some further instances demonstrating the close linkage between costal blotch colour and ground colour in cases in which the dominant factors **B** or **B**¹ are linked with the recessive factor **c** for costal blotch, since only one such case was discussed in the body of the previous paper with a brief reference in a footnote to certain results obtained immediately prior to publication. Typical pedigrees dealing respectively with the combinations **Bc** and **B**¹**c** are as follows.

♀ *brunneana* 111 × ♂ *potentillana* 104
(BcBc) | (B²CB²c)
Brood 123

16 *comparana*   18 *brunneana*
(BcB²C)   (BcB²c)

♀..........................× ♂ *proteana* 124
(BcB²C) | (B²cB²c)
Brood 144

10 *brunneana*   10 *potentillana*
(BcB²c)   (B²CB²c)
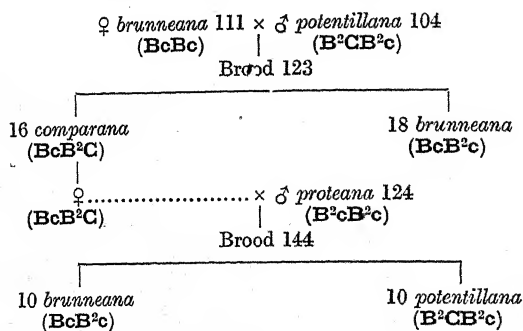
In a reciprocal cross (Brood 147) in which the female was *proteana* 124 and the male *comparana* 123, similar results were obtained, the brood consisting of nine *brunneana* and five *potentillana*.

' As regards the factor B¹, the following pedigree is typical:

♀ *latifasciana* 107 × ♂ *potentillana* 106
(B¹cB²c) | (B²CB²C)
Brood 120

7 *potentillana*   4 *fasciana*
(B²CB²c)   (B¹cB²C)

♀ *proteana* 113 ×...............♂
(B²cB²c) | (B¹cB²C)
Brood 132

9 *potentillana*   10 *latifasciana*
(B²CB²c)   (B¹cB²c)
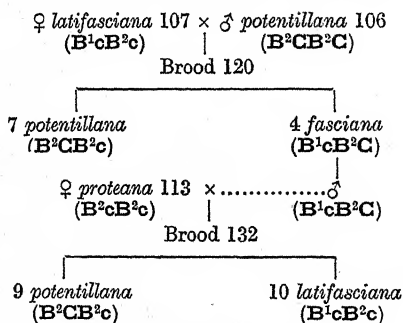
In all, eight broods comprising 158 individuals were reared of the types shown in the above pedigrees, and in no case was any crossing-over detected, and therefore the linkage is evidently very close.

Apart from the question just discussed, three further points were left open in the earlier paper and may now be dealt with, viz. the suggestion that the fasciate form of *brunneana* (Plate II, fig. 5 *B*, in the original paper) was due to the combination BcB¹c; the inheritance of the *fuscana* form; and the problem of an insect morphologically identical with *comariana* which feeds upon *Azalea* sp.

VARIATIONS WITHIN THE FORM *BRUNNEANA*.

In regard to fasciate *brunneana*, it is merely necessary to record that the suggestion has been fully borne out, and that it is possible to pick out individuals of the constitution BcB¹c without difficulty, especially so far as the Wisbech race is concerned. In the case of certain broods in which B and B¹ have been derived from *comariana* from Belgian azaleas,

the dominance of **B** over **B**[1] is less complete, and some puzzling forms almost intermediate between *brunneana* and *latifasciana* have appeared: these intermediates have light-coloured scales, as in *latifasciana*, but they can always be separated from the latter by the absence of black scaling, and by the fact that the light scales are slightly yellowish or cream-coloured, at any rate not quite white.

THE INHERITANCE OF *FUSCANA*.

In regard to *fuscana*, it may be recalled that this melanic form is only known from Lancashire, where it represents some 30 per cent. of the population from which the material containing the form was obtained. A reference to the table of results, which appears on p. 200 (which includes all broods in which *fuscana* is concerned), will show that the *fuscana* characteristics are recessive to those of all other forms tested: the dominance of the characteristics of the latter is practically complete so far as **B**[1] and **B**[2] are concerned, the chief effect of introducing *fuscana* being to produce a general dulling of the colours and in the case of *fasciana* or *latifasciana* a marked increase in the number of black scales. Where **B** is concerned, however, *e.g.* in the F_1 from a pairing between a homozygous *brunneana* and *fuscana*, the hybrids are almost intermediate between the two parents, and are curious-looking insects, superficially of dark brown colour owing to the admixture of the "tan" coloured scales of *brunneana* with the "leaden" scales of *fuscana*. In extreme cases these hybrid forms approach *fuscana*, but in general they have an appearance quite different from either. These remarks apply to hybrids in which **B** was derived from individuals from Belgian azaleas, since this factor was no longer available in moths of Wisbech origin. It is quite possible that the factor **B** from Wisbech might show more complete dominance (by analogy with the results recorded above as to the combination **BB**[1])*.

In regard to the costal blotch colour, which cannot be detected in *fuscana* owing to the general blackish suffusion, it will be noticed that all *fuscana* tested have acted as if the costal blotch were brown (due to **c**) and there is no evidence of the existence of *fuscana* with a black costal blotch.

Since the inheritance of the ground colour of other forms of *comariana* could be most easily explained on the basis of a series of multiple allelomorphs, the natural hypothesis for the explanation of the *fuscana* form

* On the evidence available it seems preferable to regard the factors designated as **B** as the same, regardless of the origin of the material, but further work might show this to be incorrect.

was to postulate a fourth number in the series—B^3—with which, so far as these experiments go, is invariably linked the recessive factor c for brown costal blotch. This explanation appears to accord reasonably well with the results, as is demonstrated by the following families.

First may be quoted a pedigree in which the various members of the series of multiple allelomorphs appear without complication of the costal blotch factors.

F_1

φ *proteana* 124 × $\male$ *fuscana* (wild)
(B^2cB^2c) | (B^3cB^3c)
Brood 160

8 *proteana*
(B^2cB^3c)

φ × $\male$

F_2

Brood 194

6 *proteana*   2 *fuscana*
($B^2cB^2c + B^2cB^3c$)   (B^3cB^3c)

$\male$ × φ *brunneana* (wild)
(B^2cB^3c) | (BcB^1c)

F_3

Brood 236

2 *brunneana*   2 *latifasciana*
($BcB^2c : BcB^3c$)   ($B^1cB^2c : B^1cB^3c$)

φ *fuscana* 213 × $\male$ *brunneana* 236 φ *brunneana* 236 × $\male$ *fuscana* 213
(B^3cB^3c) | (BcB^3c)   (BcB^2c) | (B^3cB^3c)

F_4

Brood 260   Brood 261

18 *brunneana*   7 *fuscana*   11 *brunneana*   13 *proteana*
(BcB^3c)   (B^3cB^3c)   (BcB^3c)   (B^2cB^3c)

Little comment is necessary on this pedigree; in the F_1 generation *proteana* is completely dominant, and in the F_2 *fuscana* reappears in the expected proportion of $1:3$. For the F_3 generation a *proteana* male, which subsequent broods showed to have been of the constitution B^2cB^3c, was paired with a wild *brunneana* from azalea which showed evident "fasciation," and therefore was assumed to carry the factor B^1 for "marbled" ground colour. This assumption proved correct, since the F_3 brood contained both *brunneana* and *latifasciana*. No pairings were obtained with the *latifasciana* forms, but the two *brunneana* were paired with *fuscana*, giving in one brood (260) both *brunneana* and *fuscana*, and in the other (261) *brunneana* and *proteana*, thus showing that the *brunneana* parents in 236 were of the constitution BcB^2c and BcB^3c in accordance with the hypothesis.

Next may be given a pedigree of families in which appears C, the dominant factor for black costal blotch.

$\begin{array}{c} \text{♀ } \textit{fasciana } 113 \times \text{ ♂ } \textit{fuscana} \text{ (wild)} \\ (\mathbf{B^1cB^2C}) \quad | \quad (\mathbf{B^3cB^3c}) \end{array}$

F_1

Brood 166

$\begin{array}{cc} \text{5 } \textit{potentillana} & \text{8 } \textit{latifasciana} \\ (\mathbf{B^2CB^3c}) & (\mathbf{B^1cB^3c}) \\ \text{♀} \qquad \times & \text{♂} \\ (\mathbf{B^2CB^3c}) \quad | & (\mathbf{B^1cB^3c}) \end{array}$

F_2

Brood 204

$\begin{array}{cccc} \text{5 } \textit{potentillana} & \text{6 } \textit{latifasciana} & \text{4 } \textit{fasciana} & \text{6 } \textit{fuscana} \\ (\mathbf{B^2CB^3c}) & (\mathbf{B^1cB^3c}) & (\mathbf{B^1cB^2C}) & (\mathbf{B^3cB^3c}) \\ \text{♀} \qquad \times & \text{♂} \\ (\mathbf{B^2CB^3c}) \quad | & (\mathbf{B^1cB^3c}) \end{array}$

F_3

Brood 233

$\begin{array}{cccc} \text{3 } \textit{potentillana} & \text{1 } \textit{latisfasciana} & \text{3 } \textit{fasciana} & \text{3 } \textit{fuscana} \\ (\mathbf{B^2CB^3c}) & (\mathbf{B^1cB^3c}) & (\mathbf{B^1cB^2C}) & (\mathbf{B^3cB^3c}) \end{array}$

In this pedigree the original female *fasciana* parent from Brood 113 was known to have the constitution $\mathbf{B^1cB^2C}$, C being linked with $\mathbf{B^2}$. When paired with the complete recessive *fuscana* therefore, only *potentillana* and *latifasciana* should appear in the F_1, although each would contain the *fuscana* factor $\mathbf{B^3}$. On pairing such *potentillana* and *latifasciana* the four forms *potentillana*, *latifasciana*, *fasciana*, and *fuscana* should all be produced, as was in fact the case, and a similar result was again expected and obtained in F_3. The pedigree is of some interest, not only in regard to the *fuscana* allelomorph $\mathbf{B^3}$, but also in connection with the linkage phenomena which are evidently not affected by the introduction of the *fuscana* allelomorph.

It seems unnecessary to give other pedigrees, since they are substantially similar to the above and in general agreement with the hypothesis, although perhaps attention should be drawn to Broods 186, 197, 199, 189 (see table p. 200). These broods represent the F_2 from a pairing between *proteana* and *fuscana*, which in F_1 (Brood 153) gave 14 *proteana*. Theoretically, each brood should have contained *proteana* and *fuscana* in the proportion 3 : 1, but actually the average ratio was 2 : 1 in spite of the fact that Brood 186 contained no *fuscana* at all. (The occurrence of a single *potentillana* in 199 is believed to be due to a technical error.)

THE AZALEA *COMARIANA*.

The occurrence of a moth morphologically indistinguishable from *comariana*, but of which the larvae fed upon greenhouse azalea (instead of strawberry) was noted in the previous paper, and it was hoped that there were thus two races of the species, each characterised by attachment to a certain host plant, in which case it would have been interesting

to discover the mode of inheritance of this attachment. Unfortunately, this expectation has not been realised, for experiments carried out with larvae of "azalea" parentage show that such larvae, whether newly hatched or reared for part of their lives on azalea, have a marked preference for strawberry, and only eat azalea if the latter food is absent: furthermore, the moths reared from azalea are completely fertile when paired with those fed upon strawberry. It would thus seem that the azalea *comariana* are merely a section of the species which at some time or another has got stranded in glasshouses devoted to azaleas, and those individuals which have had no opportunity of returning to strawberries have persisted under glass from generation to generation, feeding on a relatively unpalatable foodplant. In the course of the trade in greenhouse azaleas such *comariana* appear to have become distributed through most countries of Western Europe, where they are erroneously known as Hubner's species *Acalla comparana*. Under glasshouse conditions the times of appearance of the different broods have been somewhat disturbed, but in this respect little tendency to fixity of habit has been detected, and individuals of azalea parentage, on being removed from glasshouse conditions, practically regain the periodicity normal to the new environment. It may be mentioned that instances of the partial (or perhaps complete) isolation of a section of a species under glasshouse conditions are not uncommon—the so-called "Tomato Moth" (*Hadena oleracea*), which does not by preference chose any of the Solanaceae, is a familiar example, and in the azalea houses a second Tortricid moth (*T. rosana*), which is not normally an azalea feeder, is considerably more common than *A. comariana*.

FURTHER DISCUSSION.

Since the previous paper was published, 89 families, covering six or seven generations, have been reared, the seventh generation being due to the occasional interpolation of a third brood in the autumn. These families represent only 45 per cent. of the matings actually made, the remaining 55 per cent. being failures, either because no eggs were laid or because the eggs failed to produce larvae. In the families dealt with in the previous paper, 57 per cent. of the matings were successful; there has thus been a marked diminution in the proportion of successful matings, a result which does not, however, seem due to an increasing influence of factors of a lethal type but rather to the difficulty of providing satisfactory conditions for *comariana* during the autumn and winter. Thus the average proportion of successes from June matings is 60 per cent., while from August matings it is only 39 per cent.

Since the families are small—16 is the average—and the emergence of a brood is spread over 4 or 5 weeks, there is great difficulty in following any pre-arranged scheme of mating, and it is frequently necessary to pair individuals merely to keep the stock in existence, although these matings may be of such a character as to throw no light on the genetical problems involved. For this reason it has not been considered worth while to place on record here the results of all successful matings made since the publication of the previous paper. In three families single individuals of forms which, theoretically, should not be represented have appeared, but there can be practically no doubt that they are to be accounted for by errors in the manipulation of the larvae, errors which, however regrettable, are not altogether unnatural in view of the difficulty of handling and feeding young larvae of almost microscopical dimensions. Apart from these three occurrences all families comply with the demands of the hypothesis already put forward as regards the forms which should be represented, and the large broods are reasonably in accord with its numerical requirements. As a further test of the latter point, a calculation has been made of the results expected in all families not dealt with in the previous paper in which two or more forms should have appeared, and the actual results are compared with the theoretical results in the table below, the figures being given to the nearest whole number.

	Fus-cana	Pro-teana	Poten-tillana	Lati-fasciana	Fas-ciana	Brun-neana	Com-parana
Actual numbers	38	103	154	182	60	153	10
Hypothetical numbers	34	114	156	180	55	154	7
Actual numbers as percentages ...	5	15	22	26	9	22	1
Hypothetical numbers as percentages	5	16	22	26	8	22	1

Since the families summarised in this table contain 700 individuals, and since figures equally concordant were obtained from the similar analysis of 800 individuals dealt with in the earlier paper, it may reasonably be concluded that the hypothesis is sufficiently in accordance with the results to be accepted without further breeding work, and here this aspect of the investigation would have been left if it were not that evidence has recently been obtained of the existence of another melanic form differing from *fuscana*. This new form appears to have been introduced into the experiments with the strain of the species obtained from Belgian azaleas, but being a recessive it was not detected in the earlier broods. The material has, therefore, been retained, and matings have been made with a view to deciding the status of this supposed new form.

In conclusion, I must again thank Mr B. S. Williams for carrying out

the greater part of the practical work involved in the experiments, and notably for carrying on the work during my absences from the laboratory.

EXPERIMENTAL DATA.

The number after each parent denotes the brood in which it arose. W signifies an individual bred from wild larvae.

Brood	Parentage ♀	Parentage ♂	Fuscana	Proteana	Potentillana	Latifasciana	Fasciana	Brunneana	Comparana
153	Fuscana (W)	× proteana 121	—	14	—	—	—	—	—
154	Proteana (W)	× proteana (W)	2	9	—	—	—	—	—
160	Proteana 124	× fuscana (W)	—	8	—	—	—	—	—
166	Fasciana 113	× fuscana (W)	—	—	—	—	—	—	—
172	Fuscana (W)	× fuscana (W)	5	—	5	8	—	—	—
186	Proteana 153	× proteana 153	—	8	—	—	—	—	—
189	Proteana 153	× proteana 153	3	5	—	—	—	—	—
191	Fuscana 154	× fuscana 189	4	—	—	—	—	—	—
194	Proteana 160	× proteana 160	2	6	—	—	—	—	—
197	Proteana 153	× proteana 153	6	5	—	—	—	—	—
199	Proteana 153	× proteana 153	1	3	—	—	—	—	—
201	Fuscana 189	× fuscana 172	51	—	(1)*	—	—	—	—
204	Potentillana 166	× latifasciana 166	6	—	5	6	4	—	—
208	Potentillana 166	× proteana 186	—	11	17	—	—	—	—
213	Fuscana 201	× fuscana 201	13	—	—	—	—	—	—
214	Potentillana 202	× fuscana 191	—	8	—	—	—	—	—
216	Fuscana 201	× fuscana 201	5	—	—	—	—	—	—
218	Fuscana 201	× potentillana 202	—	8	4	—	—	—	—
220	Fuscana 191	× potentillana 202	—	—	10	—	—	—	—
223	Fuscana 204	× fuscana 204	4	—	—	—	—	—	—
229	Fuscana 191	× latifasciana 198	—	—	—	12	—	—	—
231	Fuscana 201	× fasciana 204	—	(1)*	3	5	—	—	—
233	Potentillana 204	× latifasciana 204	3	—	3	1	3	—	—
243	Proteana (W)	× fuscana 223	—	4	—	—	—	—	—
244	Fuscana 216	× fuscana 216	23	—	—	—	—	—	—
249	Potentillana 214	× latifasciana 229	7	—	5	4	6	—	—
250	Latifasciana 229	× latifasciana 229	6	—	—	20	—	—	—
252	Fuscana 223	× fuscana 233	28	—	—	—	—	—	—
257	Brunneana 239	× fuscana 213	—	—	—	21	—	17	—
258	Proteana 243	× brunneana 239	—	—	—	18	—	25	—
260	Fuscana 213	× brunneana 236	7	—	—	—	—	—	—
261	Brunneana 236	× fuscana 213	—	13	—	—	—	18	—
283	Brunneana 258	× fuscana 252	1	—	—	—	—	11	—
286	Brunneana 257	× latifasciana 257	1	—	—	—	—	—	—
291	Latifasciana 258	× brunneana 258	—	—	—	1	—	1	—
320	Fuscana 283	× brunneana 291	—	—	—	1	—	2	—
323	Brunneana 291	× latifasciana 291	—	7	—	19	—	20	—
						12		22	

* It is believed that the occurrence of this individual in the family was due to an error in manipulation.

SUMMARY.

1. Further experimental work has been carried out with the seven described forms of *Acalla comariana*.

2. Additional evidence is submitted in support of the hypothesis that the inheritance of the wing colour in six of these forms can be explained by assuming that the ground colour of the wing is controlled by a series of multiple allelomorphs, and costal blotch colour by two separate factors, there being close linkage between the factors for ground colour and costal blotch colour.

3. It is shown that the inheritance of the *fuscana* form can be explained by postulating an additional factor in the series of multiple allelomorphs.

ANALYSIS OF FLOWER COLOUR IN *PHARBITIS NIL.*

By YOSHITAKA IMAI.
(*Tokyo.*)

(With Plate V and Six Text-figures.)

CONTENTS.

INTRODUCTION.

THE researches in relation to the genetics of *Pharbitis Nil* have been carried on for some twenty years. Pioneers in this field are Toyama and Takezaki. Prior to them, Yasuda made crossing experiments with this plant without knowing of Mendel's paper. In the course of these researches, especially during the last fifteen years, there have been numerous publications on this subject.

The Japanese morning glory exhibits abundant variation, especially in the flower colour itself and the corolla patterns, furnishing, together with the other characters, favourable material for our investigation. Owing to the complexity of the variation exhibited, and also to the

delicacy of the flower colour when exposed to sunshine, its study is rather troublesome. Contributions to the genetics of flower colour are due especially to Hagiwara, Imai, Miyake, Miyazawa, Takezaki and Tanaka. In this paper are described the behaviour of certain genes affecting the flower colour, leaving some others for future investigation. Experimental data on the general flower colours will be omitted here.

The writer wishes to make acknowledgment to Prof. K. Miyake for his useful help and valuable advice, to Messrs B. Kanna and K. Tabuchi for their friendly assistance, and also to Prof. R. C. Punnett for his important suggestions in the preparation of this manuscript.

EXPERIMENTAL ANALYSIS WITH FLOWER COLOUR.

WHITE FLOWERS.

The genetics of the white flowers of *Pharbitis Nil* is complicated, owing to the occurrence of several forms differing in their genotypes. The common genes found in white flowers are white-1 and white-2, the dominant allelomorphs of which are complementary for the production of colour. The genetic relation of these two genes has been worked out and verified by Takezaki (1916), Imai (1921) and Hagiwara (1929 c). Another gene, white-3, which results in a white flower with white seed, was studied by Miyazawa (1923) and Imai (1927 a). The dominant allelomorph of white-3 is also complementary for the production of colour with the other two dominant allelomorphs of white-1 and white-2. Imai and Tabuchi (1929) obtained a white mutant in a duskish family, the white flower being transmitted as a dominant character to duskish. Later experiments show that this white is due to the combined effect of the genes rayed and duskish; that is, rayed results in a white flower working on the duskish component by eliminating the colour from the corolla. Hagiwara (1929 b) records the occurrence of dominant white flowers in his study. According to him, his dominant whites are produced by duplicate genes for the complete inhibition of the flower colour. A close examination of his data, however, reveals the fact that they seem to have contained some contaminated individuals which caused him to draw the conclusion for the so-called dominant whites. Hence we have as yet no completely dominant white flowers in this plant. Rayed produces a dominant white flower, but only when it works on duskish. The effect of the gene itself, however, is to reduce the colour of the corolla.

White-1. White-1 (**w1**) is on a coloured stem. The flowers and their tubes are white, notwithstanding the fact that the stems are coloured.

The stem colour of white-1 varies according to the genic composition for the flower colour, though this of course is not actually developed. Owing to their coloured hypocotyls and stems, white-1 seedlings cannot be distinguished before flowering. The writer made several intercrosses between different strains of white-1, but obtained only white-1 in F_1 and later generations. By crossing experiments white-1 is proved to be a simple recessive to normal.

White-2. The most common type of white flowers is white-2 (**w2**), which has green stems. Sometimes, however, the corolla is tinged with a very faint colour. The flower tubes are either white or coloured. White-2 may be identified in seedlings, according to their hypocotyls. White-2 is recessive to normal, and its dominant allelomorph is complementary in the production of flower colour with **w1**. The double recessive, **w1 w2**, has always a white tube and cannot be distinguished phenotypically from a white-tubed **w2**. When white-2 of coloured tube is crossed with white-1, the **w2** and **w1 w2** segregates can be distinguished by phenotypes according to their tube colours. The genotype of the so-called white-2 is not always simple, because intercrosses between white-2 give at times coloured F_1, with a 9 : 7 segregation in F_2. Its genetics require further investigation.

White-3. White-1 and white-2 bear black seeds, while white-3 (**w3**) produces white seeds. White-3 has green stems and white tubes, and behaves as recessive to normal. The dominant allelomorph of white-3 is complementary with either **w1** and **w2** in the production of flower colour. White-1 and white-2 are hypostatic to white-3.

FLOWER HUES.

According to old literature, the prototype in the flower colour of our morning glory is "dilute blue," from which many divergent forms appeared in the course of evolution under cultivation. In this paper, the flower shown in Plate V, fig. 1, is regarded as the standard or normal. In the comparison of the flower hues, it seems better to take their intense colours rather than their dilute ones, the expression of the respective colours being strong in the former. The intense blue flower is "Blackish Violet"[1] (Plate V, fig. 2), according to Ridgway's *Color Standard and Color Nomenclature* (1912). The other various hues founded in the varia-

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[1] The precise identification of the colour in general is rather difficult, owing to minor variability exhibited in a given flower colour. Therefore the identification shows the nearest colours of the respective standards. When the tube is white (tube-white) the flower colour is less reddish, or Ridgway's "Deep Blue-Violet" in this case.

tion of the flower colour are effected by several mutant genes. A number of papers have been published on flower colour, of which we may cite especially those of Miyazawa (1918, 1921), Imai (1919) and Hagiwara (1923).

Purple. The recessive gene purple (**pr**) makes blue into purplish, or Ridgway's "Cotinga Purple" in its intense variation (Plate V, fig. 3). The dominancy of blue over purple is apparently complete.

Magenta. This recessive variant blooms into magenta in colour or Ridgway's "Rood's Violet" in its intense variation (Plate V, fig. 4). Normal has brownish hairs on stems and leaves, whereas magenta (**mg**) manifests white hairs. In the white flowers, even in those having green stems, the identification of the gene magenta can be made by this trait. The hypocotyls and stems of normal are dark purplish in colour, while they are dark red on magenta plants. The double recessive, **pr mg**, is "red," or Ridgway's "Amaranth Purple" in its intense variation (Plate V, fig. 5).

Dusky. Dusky (**dy**) is a recessive gene, producing dull colour, or Ridgway's "Dark Hyssop Violet" in its intense variation (Plate V, fig. 6). In combination with **pr** and **mg**, it results in the corresponding colours; namely, Ridgway's "Deep Livid Purple" by **pr dy**, "Corinthian Purple" by **mg dy** and "Neutral Red" by **pr mg dy**.

Duskish. This variation was first described by Hagiwara (1928, 1929 *a*). The expression of duskish (**dk**) is more dilute than dusky, and it exhibits a distinct hue, viz. Ridgway's "Vinaceous Purple." The gene duskish is mutable in its property. By the combination with **pr** and **mg**, duskish also results in the corresponding variations in the flower colour. Plate V, fig. 7, is **pr mg dk**. Duskish is epistatic to dusky.

FLOWER TONES.

The variable intensity of the flower colour in combination with its variable hues leads to much complication. According to old literature, the original type in regard to the flower tones is "dilute." This shows roughly the grade of intensity, but we have several "dilute" flowers differing genetically. The writer took the most common "dilute" or Ridgway's "Bradley's Blue" (Plate V, fig. 1) as the standard type in his study, this grade being presumably regarded as the prototypic blue form. In this connection we may cite especially those papers worked out by Miyake and Imai (1920) and Miyazawa (1921).

Intense. Intense (**i**) (Plate V, fig. 2) darkens the flower colour, and behaves as a recessive to normal.

Light-1. Two light flowers due to different genes have been detected. Light-1 (**lt1**) (Plate V, fig. 8), which is recessive to intense, is linked rather closely with yellow and dusky (Imai, 1931).

Light-2. Light-2 (**lt2**) is the other lightening gene, working as recessive to intense. The gene is not located on the yellow chromosome.

Dilute. Dilute (**D**) is dominant to normal, reducing the flower tone to Ridgway's "Amparo Blue" (Plate V, fig. 9).

Tinged. Tinged (**tg**) is a recessive character to normal, reducing the flower tone to a considerable degree (Plate V, fig. 10).

FLOWER PATTERNS.

Besides self-coloured flowers containing various hues and tones as described above, we have a number of different flowers marked with particular patterns. The distribution of the colour is restricted in some regions of the corolla, the restriction, however, occurring either on its definite or indefinite parts. In the latter case, a marked variation occurs in the distribution of variegation. Several genes affecting the white margin of the corolla are known, but are not described here. Hagiwara (1926) and others have analysed the genetic composition of several flower patterns.

Speckled. Speckled (**sp**) is a recessive flower, having fine spots distributed over the corolla (Plate V, fig. 11). The character shows much variation, both qualitative and quantitative. It was first studied by Tanaka (1915), and later by Imai (1921). It restricts the extension of the anthocyanin colour in the corolla, leaving a yellowish or white background. If the speckled flowers have coloured tubes, the restriction of the pigment occurs also on them. Thus coloured-tubed white-2 flowers carrying speckled have white corollas with speckled tubes. Various colours occur with speckled restriction.

Speckled-reduced. Speckled-reduced (**sp-r**) works as a recessive modifier for speckled, and it results in almost non-spotted flowers (Imai, 1921). The speckled-reduced flowers have sometimes a few fine spots on the otherwise yellowish, pale (Plate V, fig. 12) or white background. The yellowish background colour is due to the flavone pigment, and its intensity is affected by the gene intense.

Faded. Faded (**fd**) is a recessive variation, the flower colour being faintly faded (Imai, 1921). The hypocotyls of the faded seedlings are green, except on their lowest parts, which are coloured very dilutely. When faded flower is marked with a white margin, the inner region of which is rich-coloured, there is formed a ring on the corolla (Text-fig. 1).
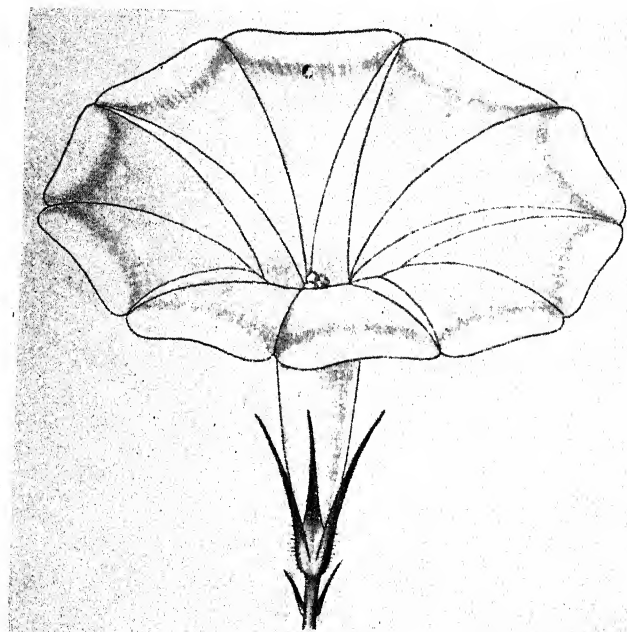
Text-fig. 1. Faded (margined).
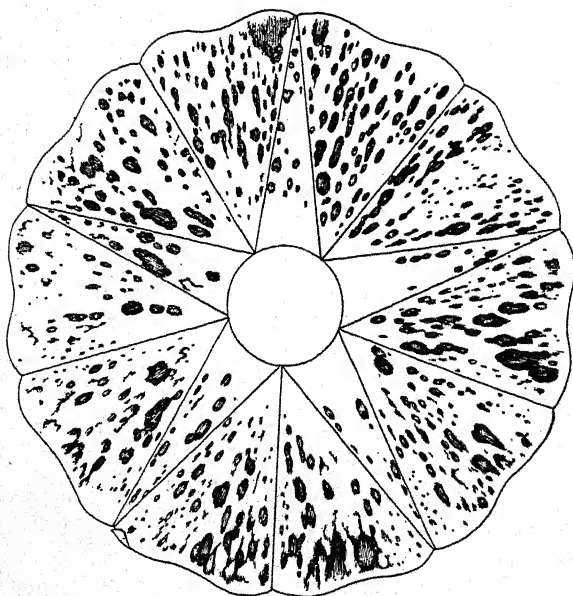
Text-fig. 2. Speckled faded.

The speckled faded flower has faded speckles with whitish centres (Text-fig. 2).

Smeary. Smeary is an intermediate form approaching faded, the flower being shaded dilutely into Ridgway's "Light Violet-Blue" in its intense blue variation. When smeary flower has a white margin, a heavy tone appears on the inner adjoining region to the white margin (Plate V, fig. 15). The stems of smeary are fully coloured. Smeary (fd^s) is recessive to normal, but dominant to faded, the three forming an allelomorphic series.
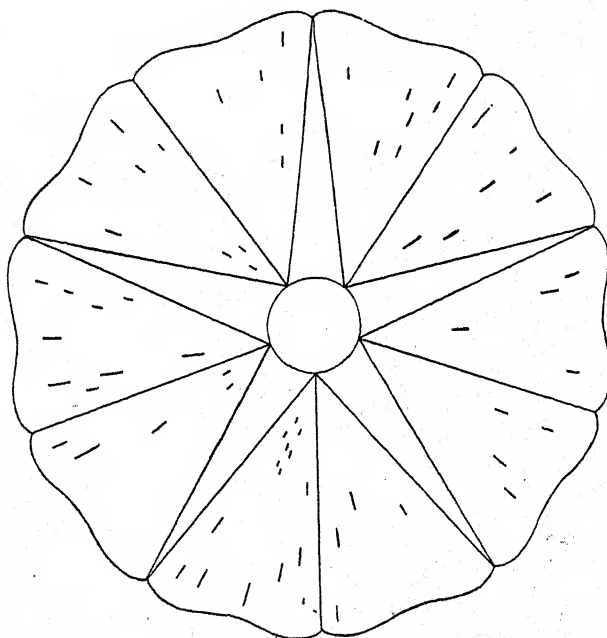
Text-fig. 3. Flecked.

Rayed. Rayed is applied to a flower having coloured rays, the colour fading away in the regions between the rays (Plate V, fig. 13). Rayed (**Ry**) is an old dominant variation, and also reappeared in the writer's material (see later). The old and new Rayed gave the same results in crossing experiments. Plate V, fig. 14, is **sp Ry**.

Flecked. Flecked flower is marked with anthocyanin flecks on the white background (Text-fig. 3). The flecks vary to a considerable extent in their size as well as in their distribution. The gene flecked (**fl**) is mutable, changing at times to its dominant fully coloured condition.

Lined. As stated elsewhere (Imai, 1929), we have a recessive variegated flower form called striped. The similar character named Lined (**Ln**), however, is dominant to normal. In Lined, deeply coloured variegation occurs on the otherwise dilutely coloured background of the corolla (Plate V, fig. 18). The amount of variegation varies to a great extent.

Striated. Striated (**sa**) has dilute flower colour, with fine stripes, the colour fading off towards the margin of the corolla (Plate V, fig. 17). Sometimes variegation occurs more plainly, distributed over the corolla. Striated gives a rather soft appearance among the variegated flowers by its delicate stripes outlined on the faintly coloured background. The gene striated is recessive to normal.

Blizzard. Blizzard flowers have deeply coloured stripes on a lightly coloured background, and frequently show sparsely scattered whitish
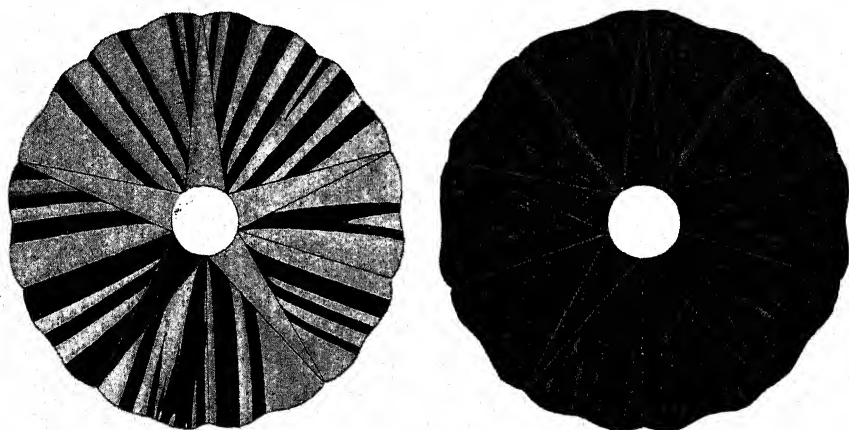
Text-fig. 4. Two forms of Blizzard, showing fluctuation.

spots (Plate V, fig. 16). Hagiwara (1926) found Blizzard to be dominant to normal, and also discovered that two complementary genes Blizzard-1 (**Bz1**) and Blizzard-2 (**Bz2**) were concerned in the production of the character. Through the interactions of these two genes the amount of variegation fluctuates to a very considerable extent (cf. Text-fig. 4), leading at times to the production of flowers which are almost without variegation.

MUTATING CHARACTERS.

Several mutating genes have been detected in the Japanese morning glory. The writer (Imai, 1927 *b*) has already studied the mutability of cream, and will now give an account of the variability of the genes flecked and duskish.

FLECKED PEDIGREE.

Source of material and inbreeding experiments.

In 1924 the writer was given a plant in a pot by his late aunt, Mrs T. Shimizu. This plant, the origin of the present study, was flecked, being characterised by white flowers with fine reddish flecks (Text-fig. 3). It showed itself to be a mosaic, bearing branches with self-coloured flowers of "red" (genetically, purple magenta) colour. The stems to which flecked flowers are attached are green, whereas those to which self-coloured flowers are attached are coloured dark red. In 1925 the writer bred the progenies from both parts, flecked and normal, on the original mosaic plant, which, however, produced rather few seeds, because of the fact that the plant was cultivated in a small pot. The offspring contained one normal and seven flecked from flecked flowers, and fourteen normal and six flecked from normal flowers on the original plant. These data, though small in number, show, first, that the gene flecked is mutable, and, second, that the normal tissues which changed from flecked are produced by vegetative mutation. These facts have been verified by the experiments made in subsequent years, and, further, showed more complication in the behaviour of flecked. Two series of pedigree culture experiments were made in 1926; one relates to the offspring of family *S*, which was derived from normal, self-coloured flowers of the original plant; and the other to family *F*, from flecked flowers. The offspring of these families are given in Table I.

TABLE I.

Data collected in the pedigree culture of flecked in 1926.

Mother plant		No. of families	+	fl	Total
Family *S*	Normal	2	55	—	55
		5	152	44	196
	Flecked	2	2	88	90
Family *F*	Normal	1	22	6	28
	Flecked	5	4	90	94

The offspring of family *S* shows that normal plants sprung from the normal part on the original mosaic plant consist of some individuals homozygous for normal and others heterozygous for flecked. The offspring of family *F*, together with those of family *S*, confirm the earlier results. The fourth generation was raised from family *F* in 1927, and Table II contains the data gathered in that year.

The amount of variegation varies widely, producing a few white

TABLE II.

Data collected in the pedigree culture of flecked in 1927.

Mother plant	Family no.	+	fl	"White"	Total
Normal	2 families	20	5	—	25
Flecked	F 5/3/1	2	46	2	50
	2	—	7	1	8
	3	2	18	—	20
	4	2	21	1	24
	5	5	71	2	78
	9	1	15*	—	16
	2 families	—	41	—	41
	Total	12	219	6	237
	Percentage	5·1	92·4	2·5	100

* One flecked plant (No. F 5/3/9/14), description of which will be found in the text, changed vegetatively to normal and was evidently a mosaic of normal and flecked.

TABLE III.

Data collected in the pedigree culture of flecked in 1928.

Mother plant	No. of families	+	fl	"Fringed"	"White"	Mosaic* F. and N.	F. and Fr.	F., Fr. and N.	Total
Normal	6	111	—	—	—	—	—	—	111
	14	208	87	—	—	—	—	—	295
	1	87	28	—	—	—	2	—	117
	1	54	20	—	2	—	1	—	77
	1	47	15	—	—	1	1	—	64
	Total	396	150	—	2	1	4	—	553
Flecked	51	—	1265	—	—	—	—	—	1265
	48	112	1722	—	—	—	—	—	1834
	9	—	408	—	15	—	—	—	423
	20	42	707	—	25	—	—	—	774
	2	—	57	—	—	2	—	—	59
	1	—	23	—	—	—	1	—	24
	2	—	42	—	—	—	—	3	45
	1	—	59	2	1	—	—	—	62
	1	—	36	—	1	—	1	—	38
	2	3	69	2	—	—	—	—	74
	7	19	379	—	—	—	10	—	408
	1	3	75	—	—	1	—	—	79
	1	2	52	1	2	—	—	—	57
	2	6	112	—	2	2	—	—	122
	1	1	18	1	—	1	—	—	21
	3	15	216	3	—	—	4	—	238
	1	6	47	—	2	1	2	—	58
	Total	209	5287	9	48	7	18	3	5581
F 5/3/9/14—F	2	38	—	—	—	—	—	40	
—S	20	10	—	—	—	—	—	30	

* F. = flecked, Fr. = "fringed," N. = normal.

individuals. The normal mutants, which appeared in the preceding generation, were revealed to be heterozygous for flecked, and the flecked families reproduced such normal mutants. In 1928[1] an extensive examination on the fifth generation was made, and the data collected are given in Table III.

The progenies of 177 plants, including 154 flecked and 23 normal flowers, were examined. Repeating the manifold variability, which was observed in the foregoing generations, flecked showed further variation, producing some "fringed" flowers in both seminal and vegetative ways. The "fringed" is a unique flower colour form, the details of which will be described later.

White variation.

Table II contains some white variants, which are entirely free from variegation throughout their plant life. During the flowering time, the writer again and again examined the flowers of the plants, which were recorded as whites, but he still found some pure whites. The whites shown in Table II are only those which were verified by daily observation. These white variants did not breed true to type in the subsequent generation, but gave nearly the same results as those obtained in the offspring of the ordinary flecked plants. In all, six white plants were used for examination, and the data thus obtained consisted of 3 normal, 137 flecked, 1 "fringed" and 4 "white." The data show the fact that the white variants appearing in the flecked families are not due to a genic change, but to a temporary variation.

Sometimes also branches bearing white flowers were observed on the flecked plants. After careful examination, such flowers were selfed, and progeny consisting of 6 normal and 61 flecked plants was obtained. This is practically the same as expected in the offspring of flecked. Therefore the white variation occurring vegetatively is also due to a fluctuating variation.

Normal mutants.

The appearance of the normal self-coloured mutants in the progeny of flecked is a recurrent phenomenon, and it is due to the inconstancy in the property of the gene, which is contained in the flecked flower. The total data, which are available from Table III, include 209 normal individuals among 5581, the proportion of the normal mutants being 3·7 per cent. From the total data collected through three generations the

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[1] On account of a trip abroad, the writer left the observations to Mr Tabuchi, who carefully recorded the flowers.

proportion is 3·8 per cent. (227 normals among 6002). In all, ten normal mutants were examined for their genotype; nine of them proved to be heterozygous for normal, and one of them was homozygous for normal. Owing to the constancy of the normal gene derived from flecked, a pure normal mutant pedigree was constructed. The mutable flecked originally might have appeared by a sporadic mutation from normal. Flecked may change into stable normal. If so the flecked mutation is of the nature of a reversion, returning to the prototype. When the normal mutant gene, however, is also mutable, as was observed in the other plants such as *Celosia* and *Plantago*, it cannot be regarded as reversion, because the two normals (one, prototype; and the other, inconstant normal mutant), even though apparently the same, are manifested by the different genes, one being non-mutable normal, and the other, mutable normal.

Mutating stages.

The mutating aspect of "yellow-inconstant" (Imai, 1930) brought the writer to the conclusion that the frequency of recurring somatic mutations varies in the different stages; that is, the mutability is high in the embryonic development, low in the post-embryonic somatogenesis, and again high in an ending stage of cell generations of leaves. This fact shows the different responsibility of the mutability of the gene "yellow-inconstant" for the different stages of somatogenesis, which works as an environment. In flecked, somatic mutations also appear in the early stage of plant cycle, some seedlings being characterised by self-coloured areas or stripes. The anthocyanin pigment, if present, is manifested very clearly on the hypocotyls. The self-coloured areas of the mosaic-flecked plants can generally be traced back to an embryonic origin, as in the case of "yellow-inconstant." The stripes or flecks appearing on corollas may be considered as due either to the propagation of the mutated cells or to the physiological differentiation of the character. The frequency of the self-coloured mutants in the flecked pedigree is 3·8 per cent. The corresponding green mutants have a proportion of 1·9 per cent. in the "yellow-inconstant" pedigree. In the latter, fine green spots appearing on leaves are evidently due to the propagation of the mutated cells. A corresponding mutation may also occur in the tissues of the flower, though actually the green cannot be visible. This relation suggests that the flecked characteristic of the corollas is due to somatic mutation, but the writer refrains from drawing any conclusion at present. Although the flecked corollas sometimes have rather broad stripes of an isolated distribution (Text-fig. 5 *a*), the flecks are generally fine.

Periclinal forms.

In "yellow-inconstant," the heterogeneous composition of bud-variations can be determined by the colour of plastids contained in the tissues, whereas, in flecked, one is handicapped by the limited distribution of the anthocyanin pigment in the tissues. For the epidermis may be coloured
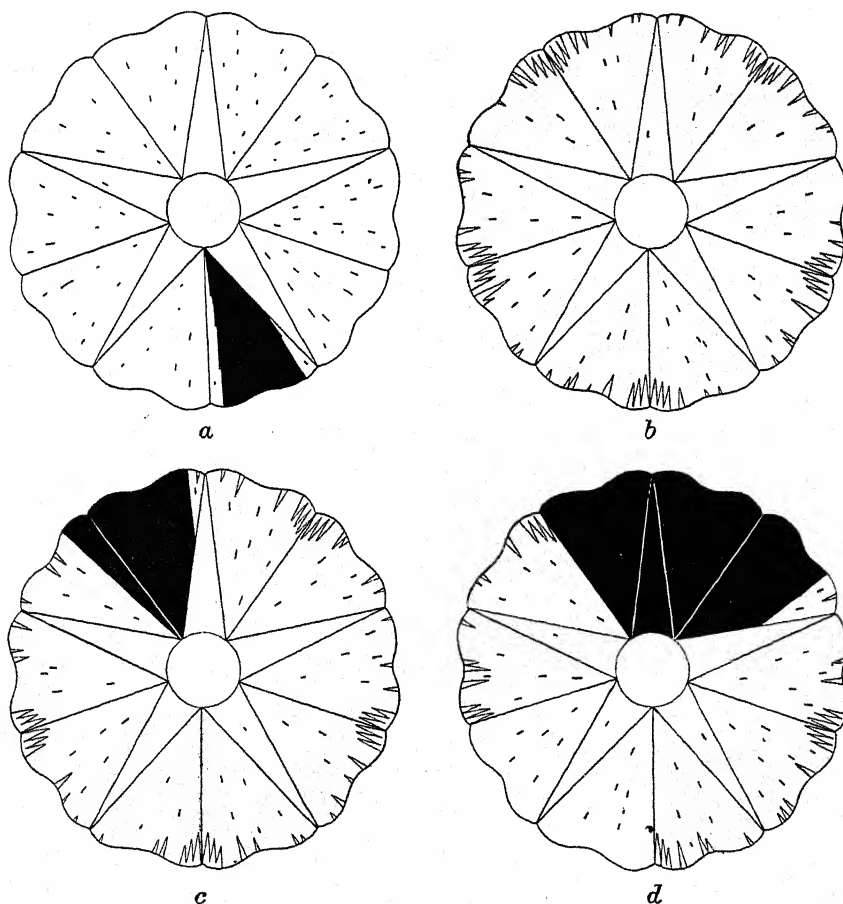
Text-fig. 5. Variations exhibited in flecked flowers. Explanation in the text.

only in the corollas, and the sub-epidermal cells only in the stems, the other tissues being free from anthocyanin pigment.

In flecked, two temporary types besides "white" are to be observed, so far as the writer's investigation is concerned. One of the two types

has self-coloured stems and dilute flowers with fringed margins; the other has green stems and self-coloured flowers of the ordinary tones. The dilute flowers are also flecked and characterised by the presence of small white areas at the margin of the corollas (Text-fig. 5 *b*). Such a form appears either as an individual, in which all flowers are characterised by this pattern, or as a bud-variation, including cases in which the "fringed" character occurs in a stripe on the corolla. The "fringed" pattern is invariably accompanied by the dilution of the flower colour and by a self-coloured stem. The offspring of three "fringed" plants contained 38 normal and 12 flecked, but no "fringed." The "fringed" branches occurring on flecked gave analogous data consisting of 29 normal and 9 flecked. The "fringed" flowers thus making their appearance were heterozygous for flecked and normal (self-coloured), the genetic aspect of "fringed" being quite the same as the self-coloured flowers of the coloured stems obtained in the flecked pedigree. Therefore, the sub-epidermal region of "fringed" became automatically heterozygous for a mutant gene which is normal. The reason why the "fringed" flowers appear on the fully coloured stems may be due to the fact that the plant is composed of heterogeneous tissues with mutant sub-epidermal regions, the epidermis remaining unchanged. The flower colour lies in the epidermal cells; therefore the flower in this case may be dilute with a "fringed" margin through the influence of the mutant sub-epidermal region, which is colourless but genetically self-coloured. The "fringed" flower gives, though not frequently, self-coloured flowers or "fringed" ones with self-coloured areas (Text-fig. 5 *c*). The self-coloured region is considered to be produced by the extrusion of the inner mutant tissues, leaving the prototypic epidermis. A flower mosaic for flecked, "fringed" and self-coloured parts (Text-fig. 5 *d*), though its occurrence is very rare, represents three possible conditions of flecked. The occurrence of small white parts, by which the flower is fringed, may be due to the fact that these marginal parts of the corolla are presumably constructed by the propagation of the epidermal cells, if a tentative suggestion is allowed at present. In chimaerical plants, *e.g.* in the white-over-green periclinal, both longitudinal sides of leaves are generally fringed with broad white regions containing mesophyll as well as veins. This suggests that the longitudinal margins of the leaves are formed solely by the propagation of the epidermal cells, and an analogous development may possibly be the case in the ontogeny of the corolla. The stem colour, however, lies in the sub-epidermal cells, therefore the stem of "fringed" is self-coloured, due to the manifestation of the mutant character.

Self-coloured flowers were rarely borne on the green stems. This is another temporary form, occurring in the flecked pedigree. On selfing two such plants, the writer obtained 1 normal and 48 flecked, but no individuals like the mother plants. Therefore, the sub-epidermal region of the variants is genetically unchanged, remaining prototypic flecked, but their epidermis is altered by mutation of flecked to normal, resulting in the self-coloured flowers by the manifestation of the mutant epidermis. The stem, however, remains green because its sub-epidermal region is unchanged.

We may therefore conclude that the two temporary forms, one having coloured stems and dilute flowers with fringed margins, and the other having green stems and normal flowers, both rarely occurring in the flecked pedigree, are periclinal chimaeras. Hence the origin of these temporary plants is somatic, being invariably due to vegetative mutation occurring in an early stage of the development of flecked plants. In "yellow-inconstant" sometimes homogeneous mutant green branches were observed, and this allows the possibility of the somatic origin of green mutants. This argument is applied to the origin of some self-coloured mutants in the flecked pedigree. The majority of the self-coloured mutants, however, may originate through mutation in the mother plants.

DUSKISH PEDIGREE.

Variability of duskish.

The duskish flower has at times very fine normal-coloured spots or flecks on the otherwise duskish background (Plate V, fig. 7). Some-
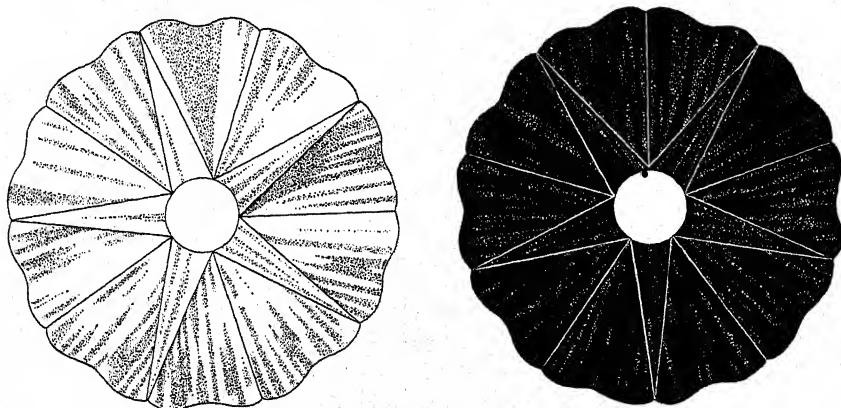
Text-fig. 6. Two "ruled" flowers, showing variability.

times, however, it blooms into a variegated corolla on the same background, or again into a self-coloured flower by the extension of the normal colour. The variegation on the duskish flowers varies to a considerable extent (Text-fig. 6). Some plants bear flowers varying in the gradation of variegation as well as plain flowers, sometimes even self-coloured ones. The variegation occurs in fine stripes of dotted constitution or of solid broad areas. In the writer's investigation on the variation of duskish, he broadly classified it into two forms; one, "plain" duskish, and the other, "ruled" duskish; the former flowers as "plain" duskish, whereas the latter flowers sometimes or almost invariably as "ruled" duskish.

Inbreeding experiments.

The original plant, No. 361 *a*, with which the present experiments were made, was "plain" duskish. Selfing the plant gave a total of 109 individuals in the subsequent generation with the numerical contents as shown in Table IV.

TABLE IV.

Data collected in the pedigree culture of duskish in 1926.

+	"Ruled"	"Plain"	Exceptional	Total
2	1	105	1	109

The offspring of "plain" duskish contained one exceptional plant with white flowers. The genetic significance of this mutant will be stated later. The subsequent generation was observed, containing progenies of 40 plants, or 37 "plain," 1 "ruled" and 2 normal. The data thus obtained are shown in Table V.

The total of "plain" duskish families contains 1·1 per cent. of normal, 5·4 per cent. of "ruled" and 93·5 per cent. of "plain," whereas the "ruled" duskish family includes 4·5 per cent. of normal, 68·2 per cent. of "ruled," and 27·3 per cent. of "plain." The difference is so remarkable that it seems to be due to a genic diversity between the two classes. From the proportions in the "ruled" family one might suppose that there occurred a simple Mendelian segregation. This, however, was not the case, which became clear when the fourth generation had been observed. In Table VI are shown the data collected in 1929[1].

The behaviour of the variability is much complicated, owing to an irregular distribution in the frequency of the segregates.

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[1] In 1928 the pedigree culture of duskish was not made, owing to the writer's absence.

TABLE V.

Data collected in the pedigree culture of duskish in 1927.

Mother plant	Family no.	+	"Ruled"	"Plain"	Total
Normal	23	24	0	9	33
	41	50	0	18	68
	Total	74	0	27	101
	Percentage	73·3	0	26·7	100
"Ruled"	33	3	45	18	66
	Percentage	4·5	68·2	27·3	100
"Plain"	17 families	0	0	348	348
	5	0	1	116	117
	8	0	6	11	17
	14	0	1	30	31
	16	0	1	8	9
	17	0	1	29	30
	27	0	1	25	26
	28	0	1	27	28
	31	0	1	25	26
	32	0	2	31	33
	34	0	1	27	28
	4 families	6	0	132	138
	4	1	11	52	64
	7	1	26	44	71
	25	2	1	87	90
	26	1	1	18	20
	29	1	5	68	74
	39	1	3	18	22
	Total	13	63	1096	1172
	Percentage	1·1	5·4	93·5	100

TABLE VI.

Data collected in the pedigree culture in 1929.

Pedigree	No. of families		+	"Ruled"	"Plain"	Total
"Ruled"—normal	3		45	6	6	57
		Percentage	78·9	10·5	10·5	99·9
"Ruled"—"ruled"	5		16	17	35	68
		Percentage	23·5	25·0	51·5	100
"Ruled"—"plain"	1		0	2	14	16
		Percentage	0	12·5	87·5	100
"Plain"—"ruled"	19		69	91	236	396
		Percentage	17·4	23·0	59·6	100
"Plain"—"plain"	15		6	38	295	339
		Percentage	1·8	11·2	87·0	100

"Ruled" variants.

In Table VI, the proportion of normal, "ruled" and "plain" in the total "plain" families (from both "ruled" and "plain" grandmother plants) is 1·7 per cent., 11·3 per cent. and 87·0 per cent. respectively. This is practically the same as the corresponding case presented in the

preceding generation (see Table V). The proportion in the "ruled" families, however, is 18·3 per cent. of normal, 23·3 per cent. of "ruled" and 58·4 per cent. of "plain." The production of normal is very great compared with that in the "plain" families. An inspection of the contents of the "ruled" families reveals the fact that the proportional difference in the segregating forms is remarkable. Under such circumstances, we cannot draw a final conclusion as to the precise nature of the variability of duskish, especially in relation to the "ruled" variant. But it is clear that: (1) "plain" gives sometimes (about 11 per cent.) "ruled" variants besides normals in its progeny; (2) "ruled" variants give a high proportion of "ruled" as well as a high proportion of normals; (3) the genetics of "ruled" variants is not simple.

Normal mutants.

Normals frequently appeared in the duskish families. Such normals proved to be heterozygotes. Tables IV and V contain the data showing this fact, and other similar results will be found in Table VII.

TABLE VII.

Offspring of normal mutants occurring in the duskish pedigree.

Family no.	+	"Ruled"	"Plain"	Total
12—1	9	1	1	11
13—1	29	1	7	37
29—1	6	4	2	12
Total	44	6	10	60
Percentage	73·3	10·0	16·7	100

In all, eight normal mutants were tested for their genotype and found to be heterozygous for duskish. The total progenies contain 163 normal, 12 "ruled" and 43 "plain," or 74·8 per cent. of normal, 5·5 per cent. of "ruled" and 19·7 per cent. of "plain." The duskish segregates occurred perfectly in accordance with a recessive ratio, their contents, however, being complicated by the irregular distribution of the "ruled" individuals. In Table VIII is collected the progeny of family No. 23, the original plant of which appeared as a normal mutant in the duskish pedigree.

Here no "ruled" duskish appeared. Owing to the small number tested, homozygous families for normality were not observed.

Rayed mutation.

In the direct progeny of No. 361 *a*, one exceptional plant, which was marked with white corollas, appeared among a total of 109, as shown in Table IV. The offspring of this variant were examined, and the results

thus obtained proved that its appearance was due to a mutation. A statement on this mutation was presented by Imai and Tabuchi (1929). At that time the writers regarded the white mutant as a dominant white, named "White-4," because of its dominant aspect to duskish, from which the so-called "White-4" appeared. Crossing experiments with this mutant

TABLE VIII.

Later offspring of normal mutants.

Mother plant	Family no.	+	"Ruled"	"Plain"	Total
Normal	23—2	13	0	7	20
	—4	12	0	4	16
	—5	13	0	4	17
	—6	13	0	6	19
	Total	51	0	21	72
	Percentage	70·8	0	29·2	100
"Plain"	23—1	1	0	8	9
	—3	0	0	18	18
	Total	1	0	26	27
	Percentage	3·7	0	96·3	100

TABLE IX.

Data collected in the Rayed mutant pedigree.

Mother plant	Family no.	"Ruled" Ry	"Plain" Ry	Ry	+	"Ruled"	"Plain"	Total
Rayed duskish	4 families	0	0	364	0	0	0	364
	9	0	1	60	0	0	0	61
	Total	0	1	424	0	0	0	425
Rayed duskish	5 families	0	0	75	0	0	30	105
	2	0	0	38	0	2	15	55
	4	0	0	59	0	1	20	80
	5	0	0	72	1	0	26	99
	15	0	0	27	0	1	10	38
	16	0	0	62	1	0	15	78
	17	0	0	22	1	0	7	30
	20	0	0	44	1	0	15	60
	22	0	1	28	1	0	9	39
	Total	0	1	427	5	4	147	584
Duskish	4 families	0	0	0	0	0	147	147
	14	0	0	0	4	0	73	77
	19	0	0	0	1	0	43	44
	21	0	0	0	1	0	10	11
	Total	0	0	0	6	0	273	279

strain to the various strains, however, revealed that the white characteristic is due to the gene Rayed working on the duskish component, *i.e.* Rayed results in a white flower with coloured tube. Therefore the name "White-4" must be abandoned. From the genetic constitution of the white mutant, it can be understood why it has coloured stems and

coloured tubes notwithstanding that it has white corollas. The rays of the corollas, however, are more or less tinged, especially on the back surface. As stated elsewhere, the progeny of the Rayed mutant consists of 25 individuals, among which 19 are duskish Rayed and 6 are duskish, indicating that the mutation brought about a plant heterozygous for a dominant Rayed gene. In 1927 the subsequent generation was observed, and the data gathered are indicated in Table IX.

The results prove the simple dominant nature of Rayed. The appearance of "ruled" duskish Rayed is due to the variable manifestation of the gene duskish.

SUMMARY.

1. The behaviour of 21 genes affecting the flower colour of *Pharbitis Nil* are described; namely, white-1 (**w1**), white-2 (**w2**), white-3 (**w3**) for white flowers, purple (**pr**), magenta (**mg**), dusky (**dy**), duskish (**dk**) for flower hues, intense (**i**), light-1 (**lt1**), light-2 (**lt2**), Dilute (**D**), tinged (**tg**) for flower tones, and speckled (**sp**), speckled-reduced (**sp-r**), faded (**fd**), smeary (**fd^s**), Rayed (**Ry**), flecked (**fl**), Lined (**Ln**), striated (**sa**), Blizzard-1 (**Bz1**). Of these genes, four are due to dominant variations. Faded and smeary are multiple allelomorphs of normal.

2. Two mutable genes, flecked and duskish, are closely studied. Flecked shows manifold variations. Sometimes normal self-coloured flowers with coloured stems appear as individual variants or bud-sports in the flecked pedigree. The occurrence of such normals is due to the mutation of the gene flecked to normal. The frequency of the occurrence of the normal mutants is 3·8 per cent. (227 normals among 6002).

3. A few "white" flowers may appear in the flecked pedigree, both as seminal and vegetative variations, but their manifestation is only temporary, fluctuating as an extreme variation of flecked. The majority of the offspring of the "white" flowers, therefore, are composed of flecked.

4. Two temporary types, besides "white," are observable in the flecked pedigree; the one has self-coloured stems and flecked, dilute-coloured flowers with fringed margins, the other has green stems and self-coloured flowers of an ordinary tone. The former type appears either as an entire plant or as a portion of a plant body. The "fringed" flower is considered to contain the mutant, sub-epidermal tissue, the epidermis remaining unchanged. The dilution of the flower colour is due to the effect of the mutant gene contained in the sub-epidermal tissue underlying genotypically colourless epidermis, because the anthocyanin

pigment is contained in the cells of epidermis in the flower. The stems of "fringed" are self-coloured, because the anthocyanin pigment is contained in their sub-epidermal region, which consists of self-coloured mutant cells. Therefore, the "fringed" type is a periclinal with a subepidermal mutant tissue. The breeding experiments prove the chimaerical nature of "fringed."

5. Another temporary form, green-stemmed self-coloured flower, is regarded as a periclinal with mutant epidermis and prototypic subepidermal cells. In this case, the flower is fully coloured, on account of its mutant epidermis; and the stem is green, on account of its unchanged, flecked sub-epidermal region. The majority of the offspring of this type, therefore, revert to flecked.

6. Duskish gives also normal plants in its pedigree, the appearance of normals being due to mutation. The frequency of such mutants is about 1 per cent. in the ordinary ("plain") pedigree. In addition to normal mutants, duskish gives at times "ruled" variants, in which the flowers are variegated. The amount of variegation varies to a marked degree. The "ruled" character is complicated, giving normal mutants as well as "ruled" variants, both in rather a high proportion.

7. Rayed mutation occurred in a duskish family. The mutant plant had coloured stems and bore white flowers with coloured tubes. Working on duskish the gene Rayed produces a white flower. Owing to the dominance of Rayed to normal, the mutant white flower is transmitted as a dominant character in the duskish family.

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EXPLANATION OF PLATE V.

(Each reduced to about two-thirds of the actual size.)

Fig. 1. Normal or standard.
Fig. 2. Intense.
Fig. 3. Purple intense.
Fig. 4. Magenta intense.
Fig. 5. Purple magenta intense.
Fig. 6. Dusky intense.
Fig. 7. Purple magenta duskish intense.
Fig. 8. Light-1.
Fig. 9. Dilute.
Fig. 10. Tinged.
Fig. 11. Speckled.
Fig. 12. Speckled-reduced.
Fig. 13. Rayed.
Fig. 14. Speckled Rayed (strain, no. 78).
Fig. 15. Smeary (margined).
Fig. 16. Blizzard-1.
Fig. 17. Striated.
Fig. 18. Lined.

PLATE V
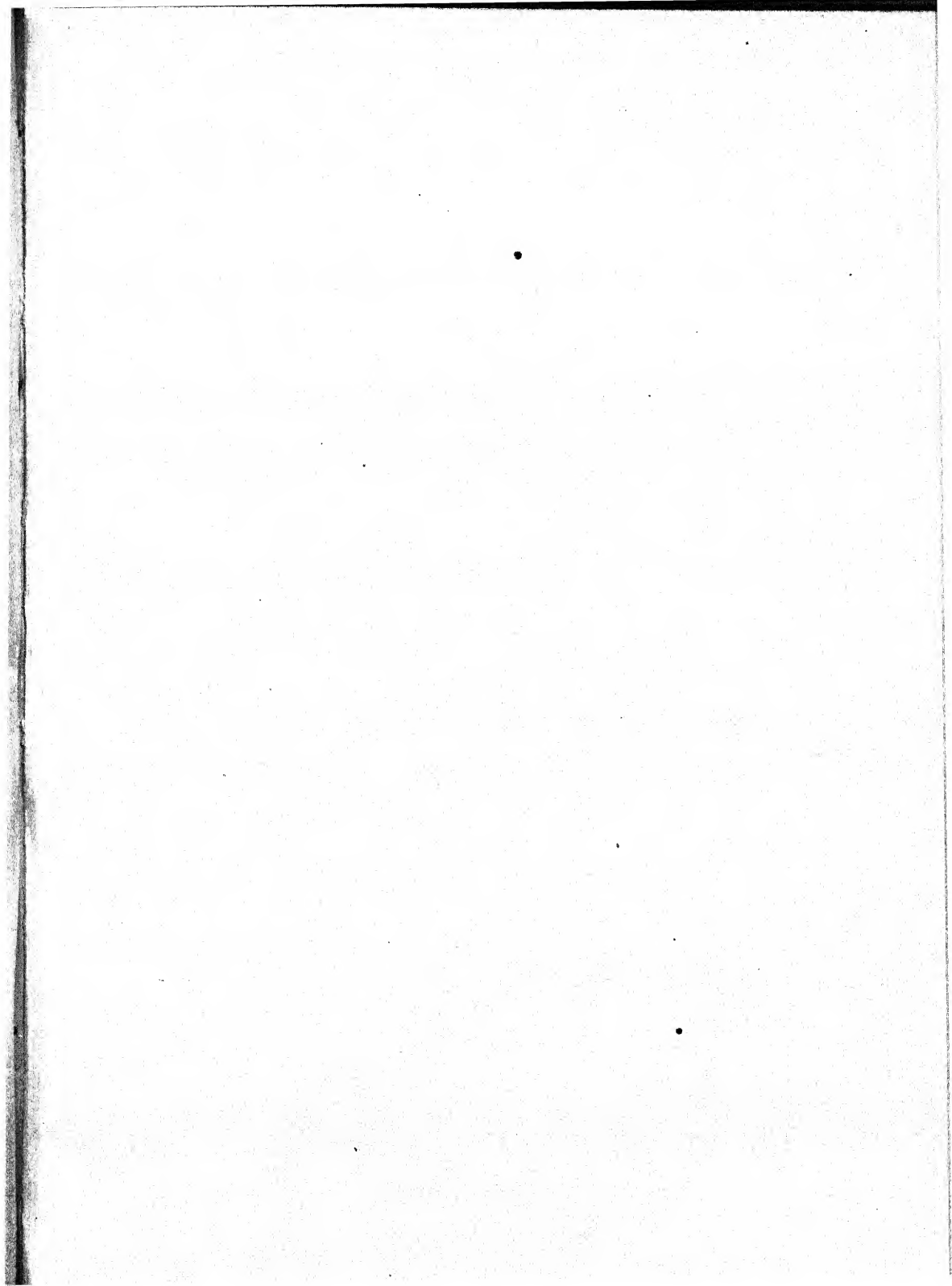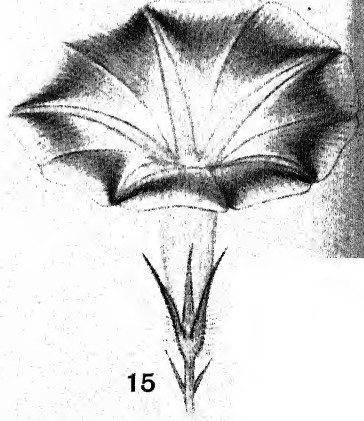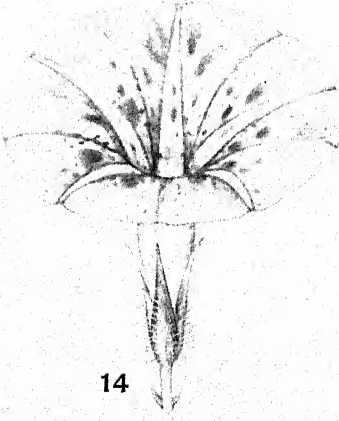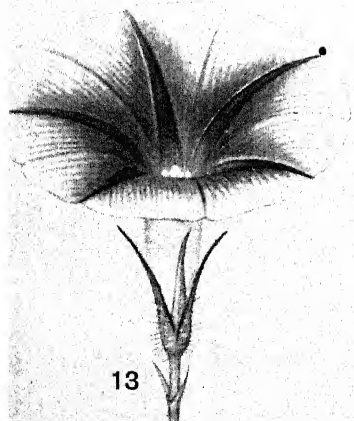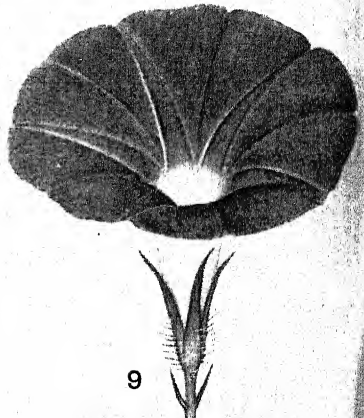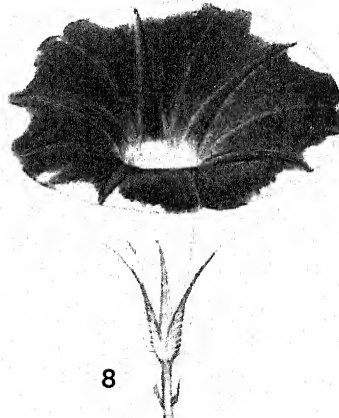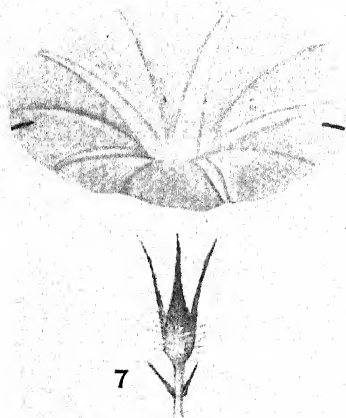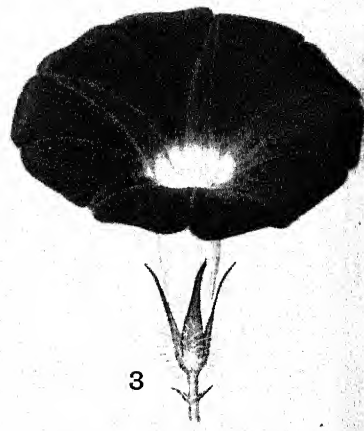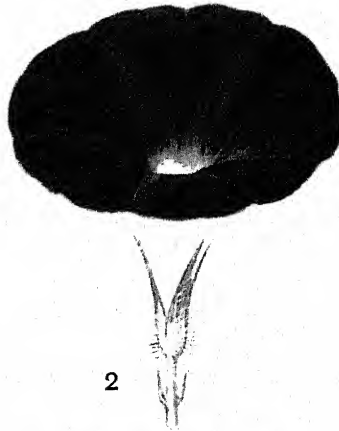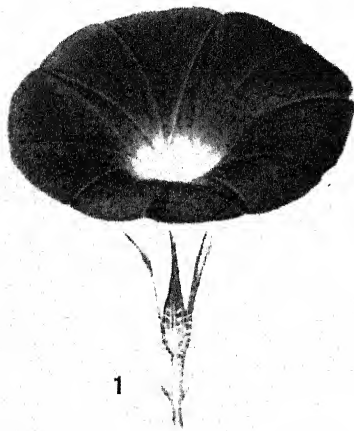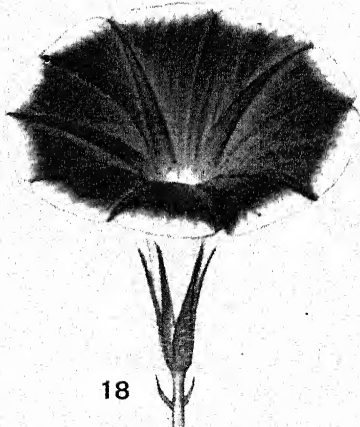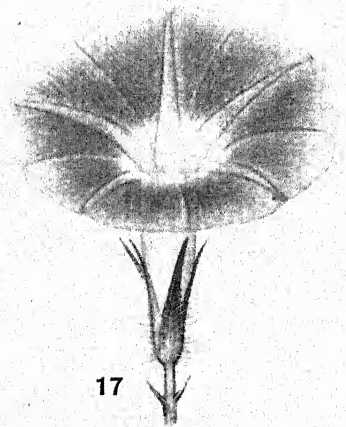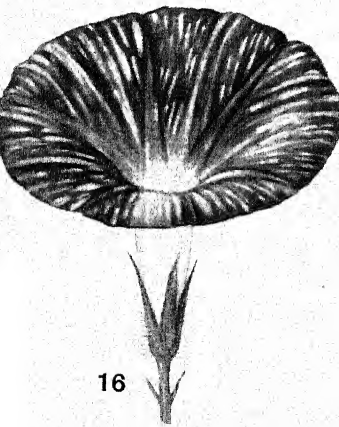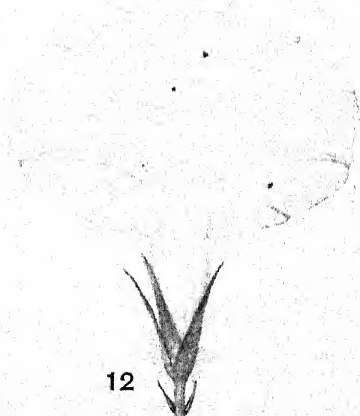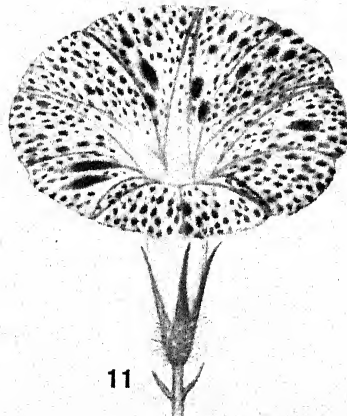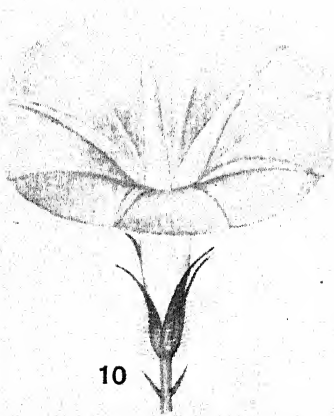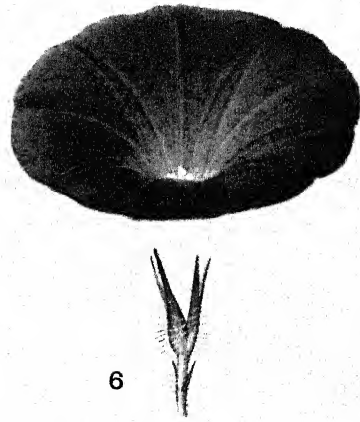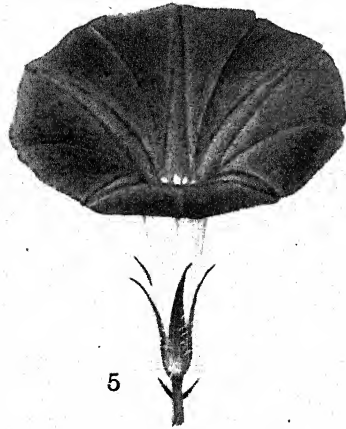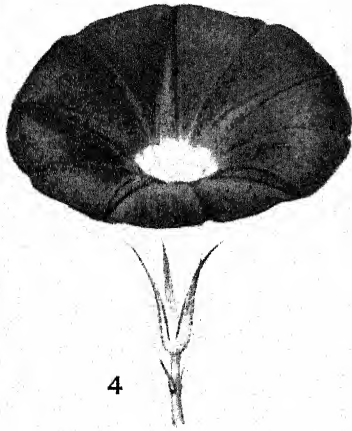
THE SEX RATIO AT THE TIME OF EMERGENCE AND THE OCCURRENCE OF UNISEXUAL FAMILIES IN THE GALL MIDGES (CECIDOMYIDAE, DIPTERA).

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(With One Chart.)

Introduction.

The chromosome theory postulates that in species reproducing sexually both sexes should occur in approximately equal numbers. On the other hand, Pictet (1926) concludes that there are three types of sex ratio. Firstly, one in which the sex ratio is 1 : 1; in this case the individuals which compose the species are descendants from an initial pair of ancestors. It is certain, he states, that this proportion is rarely realised in the natural state. Secondly, a type in which the numbers of one sex are considerably lower than those of the other. This condition arises when the offspring is derived from the crossing of races or species. Thirdly, a type in which the individuals of one of the sexes are slightly in excess; in this case the sex ratio of the populations fluctuates round 106–110 : 100. These populations, he claims, are composed of a combination of the two previous types of parents.

In the present paper are recorded several years' observations on the sex ratios that prevail among various species of Cecidomyidae. As indicated by its title the paper may be divided into two parts. The first part deals with the sex ratios of species collected in the larval stage and afterwards reared to maturity. The main object of this investigation was to obtain, as accurately as possible, the actual sex ratios that prevail in a state of nature at the time of emergence of the insects from the pupae, at which point they attain sexual maturity. It must be realised that by this time considerable mortality has probably occurred from disease, parasites and adverse weather conditions. The second part deals with the occurrence of unisexual families. In this case the insects concerned were mated, and their progeny reared, under as nearly as possible controlled conditions in order that the actual numbers of the progeny might be

obtained. Incidentally, the observations recorded show that random samples, containing large numbers of individuals, fail to disclose the occurrence of this rare phenomenon.

Part I. The sex ratio at the time of emergence.

It has been previously shown (Barnes, 1930) that, by exposing full-grown midge larvae to extra heat during the winter until the adult midges emerged, the sex ratio in the case of *D. alopecuri* was raised from 47 : 53 to 49 : 51. Similarly, if the larvae are exposed to extra heat and normal winter temperatures alternatively (twice under each condition), the ratio can be lowered from 47 : 53 to 35 : 65. The raising of the ratio, by continuous extra heat, has since found further support from the ratio of 50 : 50 obtained for *R. heterobia*, whereas under normal conditions it has never exceeded 48 : 52 and has usually been nearer 45 : 55. On the other hand, exposure to extra cold did not seem to affect the ratio of *D. alopecuri* and *R. heterobia* to the same extent.

It is very clear, therefore, that in order to study the sex ratio at emergence all the material must be subjected to the same conditions. In the breeding experiments from which figures are to be given, the conditions were as far as possible identical. Each sample was placed on soil or coconut fibre in a pot over which was placed a hurricane-lamp chimney, the upper opening of the latter being closed by means of fine gauze attached to a heavy metal ring. The pots were kept in an open-air insectary and watered from time to time. All the material was collected in the larval stage and, as might be expected, considerable mortality supervened. A certain proportion of the insects was already parasitised at the time of collection, while it is probable that some mortality occurred from other causes. This is what would prevail in a state of nature.

Dasyneura alopecuri Reuter.

The following are examples of figures obtained in 1928 of insects reared from samples of grass heads collected from different localities in 1927:

From the above figures it will be seen that there is considerable variation in the ratio for the different samples. In at least two cases, however, *i.e.* in those from Shropshire (25 : 75) and from Belfast (2) (38 : 62), the figures are in a different category to the rest.

Locality	Sex ratio	Total emergences	
		♂	♀
Rutland	42 : 58	543	755
Yorkshire	56 : 44	213	168
Shropshire	25 : 75	79	239
Derby	44 : 56	115	144
Nottingham	41 : 59	350	496
Lincoln	52 : 48	468	429
Leicestershire	43 : 57	762	1106
Total English samples*	43 : 57	3105	4114
Belfast (1)	42 : 58	657	897
Belfast (2)	38 : 62	575	938
Co. Dublin	51 : 49	213	206
Co. Tyrone	50 : 50	204	201
Co. Armagh	48 : 52	125	135
Total Irish samples*	42 : 58	1826	2491

The following figures were obtained from Aberdeen samples:

	Sex ratio	♂	♀
1929 (i)	46 : 54	5903	6807
(ii)	41 : 59	966	1408
1930 (i)	49 : 51	607	637
(ii)	43 : 57	498	656
(iii)	46 : 54	405	480
Total Scotch samples	46 : 54	8379	9988

* Figures for other samples are here included in addition to those enumerated above.

Contarinia merceri Barnes.

In this case the larvae descend to the soil for the winter months, instead of remaining in the seed cases as in the previous species. The larvae were collected while still within the grass heads and allowed to descend into the soil. The following figures were obtained in 1928 from 1927 samples:

Locality	Sex ratio	Total emergences	
		♂	♀
Shropshire	24 : 76	90	285
Yorkshire	23 : 77	260	856
Berkshire	24 : 76	44	142
Leicestershire	20 : 80	81	319
Total English samples*	24 : 76	601	1894
Caernarvon	24 : 76	52	168
Co. Wicklow	18 : 82	43	202
Co. Dublin	27 : 73	134	362
Total Welsh and Irish samples*	23 : 77	256	841

* Figures for other samples are here included in addition to those enumerated above.

It will be seen that the sex ratio for this species is markedly different from that of the preceding species.

Another species, *Contarinia tritici* Kirby, gave the following figures:

Brood	Sex ratio	Total emergences	
		♂	♀
1929 extra brood	50 : 50	118	116
1930 normal brood	47 : 53	1184	1361

Dasyneura arabis Barnes.

The species already dealt with have normally only one brood in a year, while those remaining to be considered have several broods in a year. In the case of this species the material was originally obtained from Surrey, and has since been reared in the insectary on Arabis plants. It has been the rule to cut the galls from the plants as soon as the larvae are nearly full grown and then to place the galls in lamp-glass cages as previously described. When the midges emerged a varying number was put in a cage containing Arabis plants and allowed to oviposit. The procedure was then repeated. The following figures were obtained:

	Brood	Sex ratio	Total emergences	
			♂	♀
G 0	Overwintering generation 1928–29	16 : 84	14	70
G 1	1929	—	—	—
G 2	1929	22 : 78	62	224
G 3	1929	35 : 65	155	294
H 0	Overwintering generation 1929–30	25 : 75	149	450
H 1	1930	51 : 49	164	159
H 2	1930	34 : 66	37	72

No figures are given for the third generation in 1930, because in that year it consisted of a single individual only. This omission of a generation is of frequent occurrence and is primarily due to differences of season.

Another multi-brooded species is *Dasyneura pyri* Bouché. Material of the larval stage for each brood was obtained from Devon and placed in the usual type of cage. The following figures were obtained:

Brood		Sex ratio	Total emergences	
			♂	♀
Overwintering	1928–29	28 : 72	7	18
,,	1929–30	64 : 36	72	41
First brood	1927	28 : 72	66	172
,,	1928	33 : 67	464	955
,,	1929	37 : 63	307	524
,,	1930	37 : 63	323	555
Second brood	1928	41 : 59	237	345
,,	1929	38 : 62	21	34
,,	1930	32 : 68	144	310
Third brood	1929	51 : 49	311	300

Figures have also been obtained from other localities for the second brood:

Locality	Sex ratio	Total emergences	
		♂	♀
Essex, 1926	32 : 68	143	304
Bedford, 1926	34 : 66	50	98
Nottingham, 1928	31 : 69	381	841

Another species *Stenodiplosis geniculati* Reuter has two broods a year, but figures are only available for the summer brood in 1928.

Locality	Sex ratio	Total emergences	
		♂	♀
Rutland	42 : 58	440	596
Lincoln	30 : 70	44	104
Devon	48 : 52	1444	1539
Total English samples*	46 : 54	2002	2304

* Figures for other samples are here included in addition to those enumerated above.

In the case of *Rhabdophaga terminalis* H. Lw., a multi-brooded species, material was obtained from two species of its host plant, namely *Salix alba* var. *vitellina* and *Salix coerulea*. The following are the figures:

Plant and date of sample	Sex ratio	Total emergences	
		♂	♀
S. alba var. *vitellina*			
1. viii. 28	29 : 71	432	1412
1. viii. 29	29 : 71	40	103
28. v. 29	32 : 68	79	170
S. coerulea			
1. viii. 28	57 : 43	285	219
1. viii. 29	70 : 30	28	12
28. v. 29	16 : 84	109	487

Lastly, there is *Rhabdophaga heterobia* H. Lw. The figures all relate to the overwintering brood.

Date	Sex ratio	Total emergences	
		♂	♀
1. xi. 27	42 : 58	658	914
31. x. 28	43 : 57	528	707
31. x. 29 (i)	44 : 56	208	242
31. x. 29 (ii)	48 : 52	159	170
31. x. 29 (iii)	45 : 55	111	133

The conclusions that may be drawn from the data given are as follows:

1. Some univoltine species, *e.g. Dasyneura alopecuri*, have a ratio at emergence of about equal numbers of each sex, but occasionally there is a marked diminution in the numbers of one sex.

2. Other univoltine species, *e.g. Contarinia merceri*, have a constant ratio in which one sex is markedly lower than the other, *circa* 23 : 77. This does not appear to be a generic character as was at first supposed, since *Contarinia tritici* has a sex ratio of about equal numbers.

3. In the case of multivoltine species, the sex ratio of the different broods is very variable. There is not enough evidence to conclude that each brood has a different ratio, with the tendency for later broods to approximate more nearly to equality.

4. In the case of the same species bred on different host plants, there seems to be a different ratio in each case, *e.g. Rhabdophaga terminalis.*

5. Individual samples may be affected by lethal factors killing one-half or more of the males, *e.g.* the Lincoln sample of *S. geniculati* (30 : 70) and the Shropshire sample of *D. alopecuri* (25 : 75).

Part II. The occurrence of unisexual families in the gall midges.

This feature was first reported[1] by the writer (1929) in the species *R. heterobia* and was discovered accidentally by rearing the progeny of isolated female midges. More recently R. H. Painter (1930) has recorded a similar occurrence in *Mayetiola destructor*, the Hessian Fly. C. W. Metz had previously discovered unisexual families in a closely allied group of flies, the Mycetophilidae, and the results of his work are summarised in a recent paper (1929). He has extended his investigations of this phenomenon much further than those embodied in his earlier publications. Besides these few cases of unisexual families in Diptera, a few instances are known of their occurrence in Lepidoptera. Dr P. A. Buxton has called my attention to a paper by E. Hindle (1917) wherein four types of broods of *Pediculus humanus* are recorded. These are: (1) entirely male, (2) entirely female, (3) male and female with the number of males predominating, (4) male and female with the number of females predominating. In all the foregoing instances reproduction is sexual, parthenogenesis not being involved.

In the present paper I have recorded my observations with regard to sex ratios obtained solely by breeding experiments. It has not been possible to study the problem from its cytological aspects, and for this purpose preserved material has been forwarded to Dr Metz.

Rhabdophaga heterobia H. Lw.

Having found that unisexual families occurred in *R. heterobia*, it was necessary to discover whether such families were the rule, and not the exception; whether the male or female influenced the ratio; and thirdly, whether the daughters in an all-female family produced all-male families and all-female families in equal numbers.

Accordingly 15 wild females were mated in 1930 as follows, and gave these results: 1♂ mated to 1♀ gave 0♂, 102♀ progeny. 1♂ × 1♀ gave

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[1] Eight isolated females gave the following families: 18♂, 0♀; 71♂, 0♀; 16♂, 0♀; 0♂, 46♀; 0♂, 19♀; 20♂, 0♀; 0♂, 18♀; 10♂, 0♀.

0♂, 52♀. 1♂ × 4♀♀ gave 31♂, 3♀; 39♂, 3♀; 4♂, 0♀; 1♂, 0♀. 1♂ × 4♀♀ gave 0♂, 57♀; 75♂, 0♀; 0♂ 71♀; 2♂, 73♀. 1♂ × 5♀♀ gave 0♂, 19♀; 10♂, 88♀; 11♂, 9♀; 0♂, 9♀; 36♂, 0♀. This showed that the male does not influence the determination of the sex of the progeny.

In the next brood 13 females of an all-female family were used and mated in each case to a male from an all-male family. The results were 1♂ mated to 6♀♀ gave 0♂, 13♀; 3♂, 2♀; 1♂, 8♀; 0♂, 5♀; 0♂, 59♀; 0♂, 2♀. 1♂ × 2♀♀ gave 0♂,13♀; 0♂, 4♀. 1♂ × 2♀♀ gave 0♂, 5♀; 2♂, 0♀. 1♂ × 2♀♀ gave 0♂, 39♀; –. 1♂ × 1♀ gave 14♂, 0♀. The figures are low in many cases, but it has been found that, where both sexes occur, they are generally both represented by the time five individuals have emerged.

This brood showed that females of an all-female family can give either all-female progeny, or all-male progeny, or exceptional families. It would also appear that approximately equal numbers of male-family-producing females, female-family-producing females and exceptional-family-producing females occur. The numbers of females used are too small to allow of an exact conclusion being drawn in this respect. In the first brood, out of 15 cases, 6 were all ♀ producers, 4 all ♂ producers and 5 produced families with exceptional individuals. In the second brood, out of 12 cases, 8 were all ♀ producers, 2 all ♂ producers and 2 produced families with exceptional individuals.

Chart of R. heterobia *families* 1930.

Note. All females used in generation 2 came from the first all female family in generation 1, as indicated by the arrow.
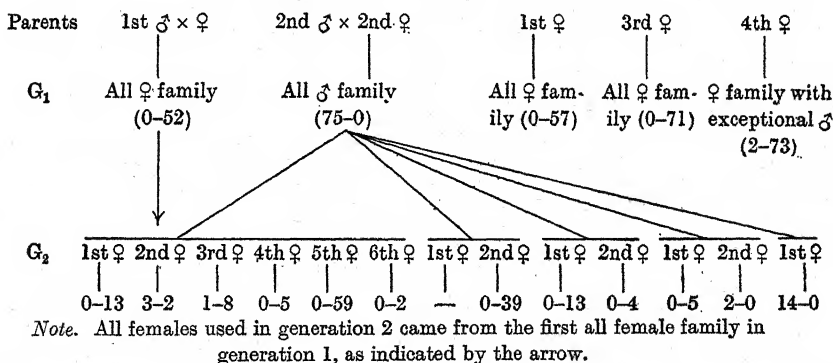
In all cases, after emergence has ceased, the galls have been examined closely, but in no case have any dead larvae or pupae been found. This would seem to preclude preferential mortality between the sexes during the larval and pupal stages. It is considered most probable that the low numbers of adults are the direct consequence of a small number of eggs being laid.

Out of all the 35 cases so far dealt with, both in the 1929 and 1930 experiments, there have been 28 cases of unisexual families and 7 cases of families in which both sexes have occurred but giving very exceptional ratios.

If we take into account only the 24 families which consisted of ten or more individuals, we have 20 unisexual families (8 male families, 12 female families); 3 nearly unisexual families (2 male families with exceptional females and 1 female family with exceptional males); and 1 anomalous or exceptional family.

It was originally thought that the crossing of individuals that emerged from different types of gall, was the cause of the unisexual progenies (cf. Doncaster, 1913): *i.e.* that different races of individuals had arisen which made the different types of galls. Certainly such crosses produced unisexual progenies, but so also did individuals mated to individuals from the same type of gall. I have since formed the opinion that the different types of galls are due to the state of the plant when the adult midges are ovipositing, and to its subsequent amount of growth.

Referring back to Part I of this paper, it will be seen that random samples of the galls of this species produce midges in the ratio of 42–48 : 58–52 and thus give no indication of this phenomenon. Similarly, if individual galls are collected in the field, both sexes usually arise from each gall. The following are the numbers of males and females emerging from individual galls: *A* 14–45, *B* 8–0, *C* 20–19, *D* 9–3, *E* 8–11, *F* 19–10, *G* 0–12, *H* 3–2, *I* 8–11, *J* 1–2, *K* 12–11, *L* 3–1. It is clear, therefore, that different females lay eggs on the same bud, and that larvae of different parents live in the same gall.

In order to discover whether other species of gall midges reproduced in this way experiments were set up using *D. arabis* Barnes, *T. oculiperda* Rübs. and *D. leguminicola* Lintner.

Dasyneura arabis Barnes.

The following figures were obtained by rearing the progeny of isolated females belonging to the second generation of 1929. All six females were mated to a single male:

	Sex ratio	Number of individuals ♂	Number of individuals ♀
First ♀	12 : 88	3	22
Second ♀	83 : 17	24	5
Third ♀	82 : 18	9	2
Fourth ♀	55 : 45	17	14
Fifth ♀	14 : 86	4	25
Sixth ♀	36 : 64	4	7

The following figures were obtained from a female of the third 1929 generation:

♀   13 : 87 ♂   2   13

In 1930 13 females of the overwintering generation were used with the following results: 1♂ mated to 3♀♀ gave 26♂, 17♀; 19♂, 11♀; 5♂, 25♀. 1♂ × 5♀♀ gave 0♂, 12♀; 6♂, 20♀; 8♂, 4♀; 6♂, 16♀; 3♂, 2♀. 1♂ × 1♀ gave 3♂, 4♀. 1♂ × 2♀♀ gave 6♂, 4♀; 13♂, 10♀; and 1♂ × 2♀♀ gave 11♂, 4♀; 1♂, 3♀.

It will be seen from the above figures that unisexual families do not occur regularly in *D. arabis* as in *R. heterobia*. But the above families can be classified according to whether there is a significant departure from the 1 : 1 ratio irrespective of percentages. If we do this there are 2 families in which males predominate, 7 families in which females predominate and 11 families which contain individuals of both sexes in approximately equal numbers.

Dasyneura leguminicola Lintner.

In similar experiments using this species, six isolated females gave the following families: 7♂, 7♀; 0♂, 1♀; 6♂, 8♀; 16♂, 15♀; 2♂, 1♀; 2♂, 1♀. It would appear from these figures that unisexual progenies are not the rule in this species.

Thomasiniana oculiperda Rübs.

This species was being studied from another view-point which involved several females being put in the same cage. The figures show clearly that it must rest under strong suspicion of producing unisexual families. The sets of figures given below represent the rearings from five breeding cages:

(1) 5♀♀ gave rise to 54 males, 57 females
(2) 5♀♀   ,,   ,,   27 ,,   21   ,,
(3) 4♀♀   ,,   ,,   0 ,,   71   ,,
(4) 5♀♀   ,,   ,,   35 ,,   77   ,,
(5) 5♀♀   ,,   ,,   18 ,, 138   ,,

The following conclusions may be drawn from Part II of this paper:

1. *Rhabdophaga heterobia* H. Lw.

(a) Unisexual families are the rule in this species, while families in which both sexes occur in approximately equal numbers are to be regarded as exceptional occurrences.

(b) The male does not directly influence the sex of the offspring of

the female with which pairing takes place. In this connection it must be mentioned that Metz (1929) has shown that exceptional males, produced in cultures, when mated to female-producing females may influence the sex-determining quality of the daughters (*i.e.* the sex of the grandchildren). These two conclusions do not contradict one another.

(*c*) Daughter offspring, in an all-female family, are able to produce all-male families, or all-female families, or families containing a preponderance of individuals of one sex or the other. Further, these four types of families are produced in roughly equal numbers.

(*d*) The prevalence of unisexual families precludes brother and sister matings and thereby ensures cross-pairing. It should, therefore, benefit the race by preventing the appearance of injurious recessive characters.

The foregoing conclusions agree, in so far as they go, with the results described by Metz.

2. *Dasyneura arabis* Barnes.

In this species there are male-producing families and female-producing families in addition to families which produce individuals of both sexes in approximately equal numbers.

Both in this species and in *Dasyneura leguminicola* Lintner the occurrence of unisexual families is not usual.

3. *Thomasiniana oculiperda* Rübs.

In this species there is strong evidence which suggests that the occurrence of unisexual families most probably obtains, but this possibility needs further inquiry.

ACKNOWLEDGMENTS.

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AMPHIDASIS BETULARIA (L.), AND ITS MELANIC VARIETIES.

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(With Plate VI.)

In 1928 some experiments were started in this laboratory in connection with melanism in Lepidoptera. Various species were used, of which *Amphidasis betularia* (L.) will be dealt with here. Of this species I had the opportunity of obtaining at the same time the type, the melanic, and an intermediate form. Since no one here had any experience in rearing large numbers of moths, I had to find out the best methods myself. This of course led to some accidents which might otherwise have been avoided. Especially was the mortality among the freshly hatched larvae far too great, only about one-third of the larvae reaching the second instar. This difficulty—probably caused by too dry conditions—has now been nearly overcome, but in the summer of 1930 another accident happened, when for some unknown reason the eggs of all the new broods but one perished when half developed, *i.e.* at a point when the embryos were already visible through the egg shells. Only one brood survived and is now hibernating, but as this brood is only a repetition of the parental brood, and the melanic stock is quite extinct, the experiments on this species will not be continued. Nevertheless some results have been obtained concerning the relation of the type form of *Amphidasis betularia* to its two melanic varieties, *i.e.* the black and the intermediate forms.

Amphidasis betularia (L.) forma *typica* (Figs. 9–12) is white with black spots and dots spread all over the wings and the body. The largest of the black spots are placed at the costal margin of the fore wings, and are parts of some indistinct and interrupted black bands running across the wings. The hind wings are often—especially in the males—lighter than the fore wings, *i.e.* they have fewer black spots and dots. The body also is white with black dots, and on the second abdominal segment it has two symmetrically arranged black spots. The number of black dots and the extension of the spots vary very much—probably on account of

genetical differences, which, however, have not been investigated. Common to all individuals referred to the type is the character of the black spots and dots of the abdomen, which are separated from each other by the white ground colour.

The "intermediate" form (Figs. 5–8) differs from the type in having so many black spots and dots that they fuse, causing the white between them to look like fine spots on a black ground. The hind wings are considerably lighter than the fore wings, especially in the males. One tuft of scales on the forehead and one on each shoulder are pure white. The abdomen is black with small white dots which were always present in my material, constituting a good character for separating the "intermediates" from the black variety[1].

Amphidasis betularia var. *doubledayaria* Mill. (Figs. 1–4) is the true melanic variety of the species. The fore wings are black without or with only few white dots, the hind wings being often somewhat greyish instead of black—especially in the males. The forehead and the shoulders show the usual tufts of white scales. The abdomen is uniformly black without any white dots.

The entomological literature contains a lot of records on *Amphidasis* rearing, most of them referring only to very few individuals. More extensive experiments on the inheritance of melanism in this species have been made by Bowater (1914) and Gerschler (1915), both of whom agree that melanism is due to a single gene, and dominant to "type." Gerschler states that he has not found any intermediate form in his material, so that the case seems to him very simple. Bowater, however, experienced more difficulty in analysing the mode of inheritance, since intermediate forms often appeared in his material, but he was not able to give any satisfactory explanation of their occurrence. The reason for his lack of success seems to be that many of the individuals referred to the intermediate form were not intermediate, but true *doubledayaria*. This is clear on comparing my material with the excellent figures given by Bowater, whose Figs. 7, 9 and 10 represent true melanics and not—as stated by him—intermediates. This mistake was probably due to two different causes: in the first place the melanic males may often have greyish hind wings (the "intermediate" specimens Figs. 7 and 9 of Bowater), and secondly some of the melanic individuals—probably the heterozygotes— have more white spots on the fore wings than usual (Bowater, Fig. 10). However, his Figs. 5, 6 and 8 certainly represent the true intermediate

[1] In Figs. 8 and 10 of Plate VI the white dots do not appear so distinctly as in the original photos.

form. Thus of course he could not succeed in tracing the inheritance of the intermediate form, and in this respect his records are of no value and will not be considered in the following discussion.

Moreover a great number of short notes on the breeding of *Amphidasis betularia* are to be found in the literature, and the reader will find below a summary of records dealing with the relation between the "intermediates" and the others. Records on broods in which only melanics and types appeared have not been considered, since the relation between these forms—as mentioned above—has been clearly worked out by Bowater and Gerschler.

TABLE I.

	Parentage		Offspring			
	♂	♀	Types	Interm.	Mel.	Totals
Orville, 1868	Mel.	× Int. (?)	—	5	3	8
Carr*, 1901–2	Type	× Int.	—	13	—	13
Schröder, 1909	Mel.	× Mel.	27	18	28	73
Schröder, 1909	Mel.	× Mel.	23	14	30	67
Miller*, 1912	Type	× Int.	50 %	50 %	—	?

* According to Bowater. I have not had access to the original paper.

Apart from Bowater no one has tried to interpret these records from a genetical point of view, but before giving my own explanation I shall pass to an account of my experiments.

TABLE II.

Table of broods.

1928	Int. ♂ × Mel. ♀		? ♂ × Type ♀
1929	Family 1		Family 2
	Type ♂ × Type ♀ Mel. ♂ × Mel. ♀		Int. ♂ × Type ♀
1930	Family 3 Family 4		Family 5

The parents of family 1 were captured in Charlottenlund on 8 June, 1928. They were sitting close together on a light wall, very probably after a successful copulation, since on the following days the female laid about 750 eggs. When I received the moths the male was already very rubbed but could still without any doubt be registered as an intermediate; the female, however, was true melanic (var. *doubledayaria*). The female parent of family 2 was captured in Geel forest on 10 June by Mr N. L. Wolff, who kindly handed it over to me. Probably this moth had already laid most of its eggs, but about 175 eggs were laid in captivity. The male parent of this family is unknown. The number of eggs in

families 3 and 4—the parents of which all belonged to family 1—were about 650 and 1120 respectively, but as seen from Table III there was a very great mortality in the broods, so that comparatively few moths emerged. The parents of family 5 were found *in copula* on 30 June, 1929, at Vilvorde, near Ordrup (all localities mentioned here are situated in North-Sealand, Denmark). The female laid no less than 3485 eggs (it was isolated in a glass box so that no error in counting is possible). Very nearly all these eggs hatched, but only about 1600 of the newly hatched larvae were kept alive for rearing. The results are given in Table III.

TABLE III.

Family	Parents ♂	♀	Types ♂	♀	Totals	Intermediate ♂	♀	Totals	Melanics ♂	♀	Totals	Totals
1	Int.	×Mel.	23	11	34	17	22	39	21	42	63	136
2	?	×Type	—	—	—	18	35	53	—	—	—	53
3	Type	×Type	13	33	46	—	—	—	—	—	—	46
4	Mel.	×Mel	—	—	—	2	8	10	15	22	37	47
5	Int.	×Type	102	67	169	94	92	186	—	—	—	355

When comparing the different tables it will be seen that the new experiments are sufficiently consistent with the earlier records, melanic specimens only occurring when one or both of the parents were melanic. Intermediate individuals, however, may be found in families of which one or both of the parents were either melanic or intermediate (Orville, 1868, Schröder, 1909, and families 1 and 4). I have not succeeded in pairing intermediate individuals to each other, but from the families 1 and 4 it appears that the gene for "melanic" must differ from that for "intermediate," and "melanic" must be dominant to "intermediate" as well as to "type." Moreover, "intermediate" must be dominant to "type" although recessive to "melanic," for type × type always produces only types. In family 2 a type female produced only intermediate offspring; this can be explained by supposing the male parent to have been a homozygous intermediate. Carr (1901–2) records a similar brood, only here the mother was the homozygous intermediate and the father the type. Miss Miller (1912) on the contrary has reared the offspring of a cross type × heterozygous intermediate, which, as in family 5, produced 50 per cent. intermediate and 50 per cent. type.

Another question now arises: Are the genes for "melanic" and "intermediate" located in different parts of the chromosomes or are they allelomorphic? In this respect family 4 may give some information, although unfortunately it is too small to supply absolutely sure results. The offspring, however, will be exactly the same when the genes are allelomorphs or when they are linked, and therefore I have only made

an attempt to find out if the genes are independent (*i.e.* situated in different chromosomes) or not.

A. If the genes are allelomorphic they must have been distributed as follows (**M** indicates the gene for "melanic," **I** the gene for "intermediate," and **t** the recessive gene for "type"):

		Offspring			
Family	Parentage	Genetical formula	Theoretical proportions	Theoretical numbers	Actual numbers
1	Mt × It	MI Mt	2	68	63
		It	1	34	39
		tt	1	34	34
2	II × tt	It	All	53	53
3	tt × tt	tt	All	46	46
4	(Two alternatives:)				
	(*a*) MI × MI	MM MI MI	3	35·25	37
		II	1	11·75	10
	(*b*) MI × Mt	MM MI Mt	3	35·25	37
		It	1	11·75	10
5	It × tt	It	1	177·5	186
		tt	1	177·5	169

B. If, however, the genes are independent of each other the results will be a little different. (The recessive gene for "type" is here indicated by small letters only.)

		Offspring			
Family	Parentage	Genetical formula	Theoretical proportions	Theoretical numbers	Actual numbers
1	Iimm × iiMm	IiMm iiMm	2	68	63
		Iimm	1	34	39
		iimm	1	34	34
2	IImm × iimm	Iimm	All	53	53
3	iimm × iimm	iimm	All	46	46
4	(Two alternatives:)				

(*a*) IiMm × IiMm

	IM	Im	iM	im			
IM	IIMM	IIMm	IiMM	IiMm	12 Mel.	35·25	37
Im	IIMm	IImm	IiMm	Iimm	3 Int.	8·81	10
iM	IiMM	IiMm	iiMM	iiMm	1 Type	2·94	0
im	IiMm	Iimm	iiMm	iimm			

(*b*) IiMm × iiMm

	IM	Im	iM	im			
iM	IiMM	IiMm	iiMM	iiMm	12 Mel.	35·25	37
im	IiMm	Iimm	iiMm	iimm	2 Int.	5·875	10
iM	IiMM	IiMm	iiMM	iiMm	2 Type	5·875	0
im	IiMm	Iimm	iiMm	iimm			

5	Iimm × iimm	Iimm	1	177·5	186
		iimm	1	177·5	169

From this it appears that in case A family 4 should not include any types, while in case B types may occur in the proportions of 1 : 15 or 1 : 7 respectively. No types were obtained, but since the actual numbers are small it may be asked whether this is not merely a chance result, the genes being nevertheless independent. The chance that no type occurs among the 47 individuals which constitute the whole family will be in the case B (*a*): $(15/16)^{47} = 0.04779$. That is, if this experiment (with 47 individuals) was made 100 times, about 95 cases would include types, and in less than 5 cases no types would appear. In the case B (*b*) the chance will be so small (about 0·2 per cent.) as to be left out of consideration. It must be borne in mind, however, that in family 4 the mortality was very considerable, thus making it possible that individuals of certain gene constitutions were selected, though nothing supporting such a supposition was observed. These broods do not therefore give any final proof of the theory of interdependent genes, but by far the greatest probability is that the genes are either allelomorphic or linked.

However, as a result of the experiments it has been shown that in *Amphidasis betularia* two genes occur, which influence the pigmentation of the wings and body, and moreover some other factors seem to cause much smaller differences in pigmentation. In this respect *Amphidasis betularia* resembles the case of *Lymantria monacha*, which Goldschmidt worked out some years ago (1921).

SUMMARY.

Of *Amphidasis betularia* (L.) three forms occur in nature: the type, the true melanic variety *doubledayaria* Mill., and a well characterised intermediate form, the occurrence of which is due to a single gene, which is dominant to the gene for "type" but recessive to "melanic." The intermediates are especially characterised by the white dots on black ground of the abdomen.

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[1] According to Bowater.
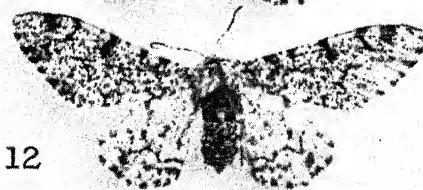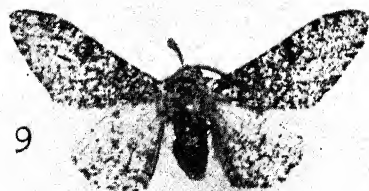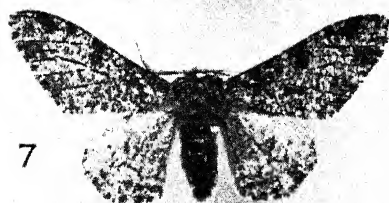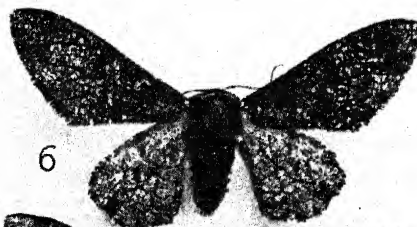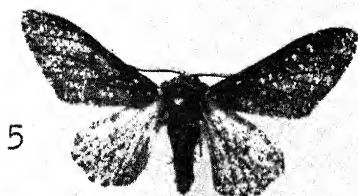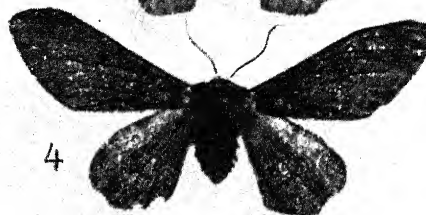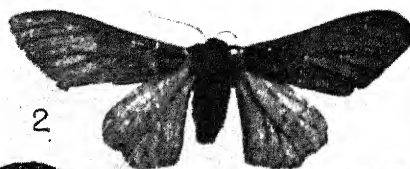
INHERITANCE OF SEX, COLOUR AND HAIRINESS IN THE RASPBERRY, *RUBUS IDAEUS* L.

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(With Plates VII and VIII and Two Text-figures.)

CONTENTS.

INTRODUCTION.

In the year 1919 Mr E. A. Bunyard drew our attention to a rogue which commonly occurs in plantations of the raspberry variety Superlative. The leaves of Superlative are characteristically curled, and the surface convex and rugose, whilst those of the rogue are comparatively flat with an even surface. Other differences between the rogues and the type occur and are described later.

The frequent occurrence of these flat-leaved rogues, and the many known examples of plants with curled and rugose leaves giving forms with flat and even leaves from endogenous buds, suggested that the rogues might arise as root sports from Superlative. To test this we raised a considerable number of plants from small pieces of the roots of Superlative. These plants were in all respects identical with the type. Moreover, it is probable that an orderly or periclinal chimerical constitution could not be maintained in the raspberry as in many perennial plants, owing to its form of asexual reproduction. The growth of the raspberry dies in its second year, and although exceptions occur, the new canes generally arise endogenously from the roots. In view of this, and from the genetic results presented in the present paper, we have concluded that the

rogues do not arise somatically from the type, but that they probably arise from chance seed germinating among the rows.

A rogue, somewhat analogous to the Superlative rogue, was found by Mr N. H. Grubb, of the East Malling Research Station, in the variety Norwich Wonder, and we are indebted to him both for the type and rogue plants of this variety used in these experiments.

In 1920 a series of breeding experiments was begun with the above forms, and more recently the varieties Lloyd George and Pyne's Royal were included. The following is a preliminary account of the investigations.

<div align="center">DESCRIPTION OF MATERIAL.</div>

In Table I are descriptions of the varieties and forms used in the experiments, but only those characters which have been genetically studied are described.

All the cultivated varieties of the raspberry which we have examined are hermaphrodite, but three other sexual forms have appeared in our experiments, namely male, female and neuter. A heterozygous sex condition appears to be common among cultivated raspberries[1].

The male plants have *obtuse*, downfolded and undivided leaves on the two-year-old fruiting canes, and on the young, one-year-old canes the leaves have only three lobes and very short petioles. They are quite distinct from the *normal* lobed and spaced leaves of the hermaphrodite and female plants. The obtuse character of the males is also expressed in the flower buds (Plate VII, fig. 1 A, B, C).

The morphological characters of the leaves and the sex condition of these male plants approximates to *Rubus idaeus obtusifolius* Willd., although according to Focke (1911) *obtusifolius* forms occasional seeds from which weakly plants have been raised. We have closely observed the male plants which have appeared in our experiments: they are all strictly male, on no plant has a single ovule developed.

The female organs of the hermaphrodites are always well developed. In the females the male organs are considerably suppressed and have no functional pollen; only rudiments of the stamens remaining. Among the hermaphrodites the male organs are variable. They all have viable pollen, but variation occurs in the length of the filaments of the stamens. In the males, the female organs are completely suppressed, but the

length of the filaments of the stamens is also variable as in the herma-
phrodites. In the neuters which appeared in family 1/25 both stamens
and styles are suppressed and are non-functional.

It will be seen from Table I and the results which follow that in
addition to the correlation between obtuse foliage and the male sex
condition there is also a correlation between the colour of the spines and

TABLE I.

Variety	Leaves	Growth	Spines	Flowers	Fruit
Superlative (Type)	Normal, curled, rugose	Sub-glabrous	Deep red	⚥⚥⚥	Red
Superlative (Rogue A)	Normal, nearly flat	,,	,,	⚥	Red, smaller than type
Superlative (Rogue B)	,, ,,	,,	,,	⚥⚥⚥	,,
Norwich Wonder (Type)	Normal, curled	Hairy	,,	⚥	Red
Norwich Wonder (Rogue A)	Normal, nearly flat	,,	,,	⚥	Red, smaller than type
Lloyd George	Normal, flat	,,	Very deep red	⚥⚥⚥	Red
Pyne's Royal	Normal, curled, rugose	Sub-glabrous	Red	⚥	,,
Seedlings:					
ex Superlative (Type)					
1/20/80	Obtuse	,,	Tinged	♂♂♂♂	—
1/20/125	Normal	,,	Deep red	⚥	Red
2/25/63	Obtuse	,,	Tinged	♂♀♂♀♂	—
2/25/2–59	,,	,,	Green	♂	—
1/25/1–47	Normal	,,	Tinged	⚥♂⚥♂⚥	Red
3/25/3–107	,,	,,	,,	⚥	,,
2/27/1–1	,,	,,	,,	⚥	,,
ex Superlative (Type) × Rogue A					
11/20/9–41	,,	,,	Green	⚥	Yellow
ex Norwich Wonder (Type)					
7/20/6–47	Obtuse	Hairy	,,	♂	—
1/23/14–36	Normal	,,	,,	⚥♂	Yellow
ex Lloyd George					
9/27/5–3	,,	,,	Very deep red	⚥	Red
9/27/5–8	,,	,,	,,	⚥♂⚥♂⚥	,,
9/27/3–11	,,	Sub-glabrous	,,	,,	,,
9/27/6–7	,,	Hairy	Green	⚥	Yellow
9/27/5–5	,,	,,	,,	⚥	,,

the colour of the fruit. The red and tinged spined forms always have red
and the green spined forms yellow fruits.

The distribution of hairs in the raspberry is rather peculiar; the
sub-glabrous character can only be seen in the young canes. The old
fruiting canes are uniformly hairy, but hairy and sub-glabrous are well-
defined characters and genetically distinct. They have been described in
detail by Grubb (1922).

BREEDING INVESTIGATIONS.

The results of the breeding investigations have shown that two pairs
of factors determine sex, two colour and one hairiness. **T** is a colour
factor which produces anthocyanin in spines and fruits. **P** intensifies the

colour of the spines. **MF** individuals are hermaphrodite; **Mf** male; **mF** female and **mf** neuter. In **Mf** and **mF** plants the female and male organs respectively are suppressed. In the absence of **H** the growth of the young canes is sub-glabrous. The phenotypes therefore are as follows:

PT fruits red, spines red,
T   fruits red, spines tinged,
P   fruits apricot? spines green,
pt fruits yellow, spines green,
FM flower ⚥,
F   flower ♀,
M   flower ♂,
mf flower neuter,
H   growth hairy,
h   growth sub-glabrous.

In the following tables the genetic constitution of the parental forms is denoted. In a few cases it is not possible to determine whether the

TABLE II.

Family no.	Leaves... Parents	Normal Red	Tinged	Green	Obtuse Red	Tinged	Green	Total Normal	Obtuse	Total Red	Tinged	Green
/28	11/20/9–41 (selfed)	—	—	37	—	—	—	37	—	—	—	37
	P–tt F–MM	—	—	*37*	—	—	—	*37*	—	—	—	*37*
/28	1/23/14–36 (selfed)	—	—	67	—	—	—	67	—	—	—	67
	pptt F–MM	—	—	*67*	—	—	—	*67*	—	—	—	*67*
	Totals: Observed	—	—	104	—	—	—	104	—	—	—	104
	Expectation	—	—	*104*	—	—	—	*104*	—	—	—	*104*
'29	2/27/1–1 (selfed)	—	94	—	—	33	—	94	33	—	127	—
	ppTT FfM–	—	*95·25*	—	—	*31·75*	—	*95·25*	*31·75*	—	*127*	—
'28	1/25/1–47 (selfed)	—	167	40	—	46	8	207	54	—	213	48
	ppTt FfM–	—	*146·8*	*48·9*	—	*48·9*	*16·3*	*186·75*	*62·25*	—	*186·75*	*62·25*
'28, 5/29	3/25/3–107 (selfed)	—	16	8	—	3	1	24	4	—	19	9
	ppTt FfM–	—	*15·75*	*5·25*	—	*5·25*	*1·75*	*21*	*7*	—	*21*	*7*
	Totals: Observed	—	183	48	—	49	9	231	58	—	232	57
	Expectation	—	*162·54*	*54·2*	—	*54·2*	*18·06*	*216·75*	*72·25*	—	*216·75*	*72·25*
29	1/25/1–47 × 2/25/2–59	8	26	33	13	7	27	67	47	21	33	60
	ppTt FfMm ×Pptt ffMm	*14·25*	*14·25*	*28·5*	*14·25*	*14·25*	*28·5*	*57*	*57*	*28·5*	*28·5*	*57*

parents are homozygous or heterozygous for certain factors, *e.g.* in family 3/28 it cannot be determined whether the parent is homozygous or heterozygous for the factor **F**.

(a) The inheritance of colour.

Table II shows the results from selfing and crossing plants with green and tinged spines. Green-spined forms may be of three kinds, namely

PPtt, Pptt and **pptt,** and each breeds true to green in the absence of the colour-producing factor **T.**

The fruits of the green-spined plants are of two kinds, (1) clear yellow, and (2) apricot. The apricots are faintly tinged red, especially when over-ripe, and although we are not at present certain, we suspect that the apricots are the green-spined forms which carry the intensifying factor **P.** These differences are not of course evident in the male and neuter plants, and whether they carry **P** or not can only be shown by appropriate crosses. Further work is in progress to determine whether these genetically different greens are phenotypically distinct.

The 127 plants in family 7/29 (**TT** selfed) are all tinged, and families 2/28 and 6/28 (**Tt** selfed) give both tinged and green as expected, but there is a deficiency of green-spined forms. In the other tables it will be noted that the proportion of plants with green spines is commonly below expectation.

As the factors **P** and **T** are complementary in their effect on the phenotype, it is obvious that reds will appear in crosses between tinged and certain greens. This is demonstrated in family 4/29 (**ppTt × Pptt**) where an approximation to expectation is obtained, the observed results being

<div align="center">red 21 : tinged 33 : green 60,</div>

and the expectation

<div align="center">red 28·5 : tinged 28·5 : green 57.</div>

Table III gives the results from selfing and crossing **PPTt** red-spined plants. No tinged plants occur, as all the progeny carry **P.** In the presence of **T** they are red; in its absence green. There is a deficiency of greens in the six families raised, the total observed being, red 309 : green 86, and the expectation, red 296·25 : green 98·75.

In Table IV are given the results from families raised from red **PPTt** back-crossed with **PPtt.** The ratio, red 136 : green 131, is almost equality as expected.

The remainder of the crosses and selfs involve the varieties Superlative, Norwich Wonder and their rogues, all of which are heterozygous for both **P** and **T.** Derivatives of these forms have also been used, and the results obtained are given in Tables IV, V and VI. Table V shows the results from **PpTt × ppTt.** The proportion of tinged plants in family 1/28 is much higher than expected, but in family 5/28 although the numbers are small the results agree closely with expectation.

The results from **PpTt × PpTt** and **PpTt** forms selfed are presented in Table VI. In these families there is considerable departure from

expectation. Too many red- and too few green-spined forms occur. In families 3/25 and 2/25, **PpTt** × **ppTt**, and 1/25, **PpTt** × **PpTt**, the same divergence occurs.

TABLE III.

Family no.	Parents	Spines Red	:	Green	Sex ⚥
9/27	Lloyd George (selfed)	81	:	15	96
	PPTt MMFF	*72*	:	*24*	*96*
6/29	9/27/5–3 (selfed)	37	:	8	45
	PPTt MMFF	*33·75*	:	*11·25*	*45*
8/29	9/27/5–8 (selfed)	68	:	20	88
	PPTt MMFF	*66*	:	*22*	*88*
10/29	9/27/3–11 (selfed)	13	:	4	17
	PPTt MMFF	*12·75*	:	*4·25*	*17*
9/29	Pyne's Royal (selfed)	41	:	20	61
	PPTt –MFF	*45·75*	:	*15·25*	*61*
3/29	Lloyd George × Pyne's Royal	69	:	19	88
	PPTt MMFF × PPTt –MFF	*66*	:	*22*	*88*
	Totals: Observed	309	:	86	395
	Expectation	*296·25*	:	*98·75*	*395*

TABLE IV.

Family no.	Parents	Spines Red	:	Green	Sex ⚥
1/29	Lloyd George × 9/27/6–7	85	:	95	180
	PPTt MMFF × PPtt MMFF	*90*	:	*90*	*180*
2/29	Lloyd George × 9/27/5–5	51	:	36	87
	PPTt MMFF × PPtt MMFF	*43·5*	:	*43·5*	*87*
	Totals: Observed	136	:	131	267
	Expectation	*133·5*	:	*133·5*	*267*

TABLE V.

Family no.	Parents	Leaves... Spines...	Normal Red	Tinged	Green	Obtuse Red	Tinged	Green	Leaves total Normal	Obtuse	Spines total Red	Tinged	Green
1/28	Superlative × 2/25/63 (**PpTt MmFf**) × (**ppTt Mmff**)		25 *33·5*	56 *33·5*	21 *24·4*	23 *33·5*	37 *33·5*	17 *24·4*	102 *89·5*	77 *89·5*	48 *67·1*	93 *67·1*	38 *48·8*
5/28	Superlative × 2/25/2–59 (**PpTt MmFf**) × (**Pptt Mmff**)		2 *3·9*	2 *1·3*	6 *5·2*	4 *3·9*	2 *1·3*	5 *5·2*	10 *10·5*	11 *10·5*	6 *7·9*	4 *2·6*	11 *10·5*

In Table VII the results from families raised from Norwich Wonder and its rogue are summarised. The proportion of red- and tinged-spined forms in families 8/20 and 9/20 (**PpTT** × **PpTt**) closely agrees with the expectation, *i.e.* red 115 : tinged 40 observed, and red 116·25 : tinged 38·75 expected.

(b) The inheritance of sex.

Our records relating to the inheritance of sex are at present of two kinds, (a) those from families which have flowered, (b) those from families which have not reached maturity, but which can to some extent be preliminarily recorded by their foliage. The hermaphrodites and females always have normal foliage, and the males obtuse foliage.

Reference to Tables III and IV shows that Lloyd George and its derivatives (by selfing) have given a total of 513 plants all hermaphrodite. Lloyd George is therefore a homozygous dominant, **MMFF**. Superlative Rogue B is also **MMFF**, giving 31 hermaphrodites from selfing and 67 hermaphrodites when crossed with Superlative type **MmFf**. Superlative Rogue A × Superlative type gave 73 hermaphrodites and therefore Rogue A must also be **MMFF**.

A very close agreement with expectation was found in the following families. Family 3/25, ⚥ **MmFf** × ♂ **MMff** gave 71⚥ : 69♂. Family 2/25, ♀ **mmFf** × ♂ **MMff** gave 68⚥ : 71♂. In both families equality was expected. In family 7/20 ⚥ **MMFf** selfed, there is a deficiency of males, 128⚥ and 30♂ occurring where 118·5⚥ and 39·5♂ were expected. Family 1/25, ♀ **mmFf** × ⚥ **MmFf** gave 20⚥, 24♀, 11♂ and 3 neuter, when expectation was 21·7⚥, 21·7♀, 7·2♂ and 7·2 neuter. The female class includes 5 weak females which set only occasional fruits and drupels. Their styles are shorter and thinner than normal. These weak females can be recognised from the neuters by their normal leaves (see Plate VII, fig. 1 E). The neuters are absolutely sterile on both sides, their flower buds never develop properly, the petals are greatly reduced and the buds only open partially.

In family 1/20 only three sex forms were recorded, where four were expected. This was, however, the first family raised, and it is possible at this early stage of the investigations that a few of the plants recorded as males may have been neuters.

The remainder of the families have not yet flowered, but the segregation of normal and obtuse foliage is of interest and as shown in the tables in most cases agrees fairly well with expectation.

(c) Inheritance of hairiness.

The records relating to hairiness are summarised in Table VIII. The segregation of this character in certain individual families is also given in Table VI. Sub-glabrous **hh** forms, selfed or intercrossed, have always bred true. In most of the families raised from heterozygous **Hh** forms

TABLE VI.

Parents	Red ☿	Red ♀	Red ♂	Tinged ☿	Tinged ♀	Tinged ♂	Green ☿	Green ♀	Green ♂	Total sexes ☿	Total sexes ♀	Total sexes ♂	Total colour Red	Total colour Tinged	Total colour Green
Superlative (selfed) PpTt MmFf	62	8	15	24	5	9	11	0	0	97	13	24	85	38	11
Expected	*42.4*	*14.1*	*14.1*	*14.1*	*4.7*	*4.7*	*18.8*	*6.2*	*6.2*	*75.3*	*25.1*	*25.1*	*75.3*	*25.1*	*33.5*
Superlative × Superlative Rogue B; PpTt MmFf × PpTt MMFF	14	—	—	4	—	—	3	—	—	21	—	—	14	4	3
Superlative Rogue B × Superlative; PpTt MMFF × PpTt MmFf	30	—	—	8	—	—	8	—	—	46	—	—	30	8	8
Superlative × Superlative Rogue A; PpTt MmFf × PpTt MMFF	18	—	—	4	—	—	3	—	—	25	—	—	18	4	3
Superlative Rogue A × Superlative; PpTt MMFF × PpTt MmFf	33	—	—	9	—	—	6	—	—	48	—	—	33	9	6
Superlative Rogue B (selfed); PpTt MMFF	27	—	—	2	—	—	2	—	—	31	—	—	27	2	2
Totals: Observed	122	—	0	27	0	0	22	0	0	171	0	0	122	27	22
Expected										*171*			*96.2*	*32.1*	*42.7*
Superlative × 1/20/80; (PpTt MmFf) × (ppTt MMff)	34	0	39	27	0	18	10	0	12	71	0	69	73	45	22
Expected	*26.3*	*0*	*26.3*	*26.3*	*0*	*26.0*	*17.5*	*0*	*17.5*	*70*	*0*	*69.5*	*52.5*	*52.5*	*35.0*
1/20/125 × 1/20/80; (ppTt mmff) × (ppTt MMff)	34	0	44	22	0	16	12	0	11	68	0	71	78	38	23
Expected	*26.0*	*0*	*26.0*	*26.0*	*0*	*26.0*	*17.4*	*0*	*17.4*	*69.5*	*0*	*69.5*	*52.1*	*52.1*	*34.8*
Totals: Observed	68	0	83	49	0	34	22	0	23	139	0	140	151	83	45
Expected	*52.3*	*0*	*52.3*	*52.3*	*0*	*52.3*	*34.9*	*0*	*34.9*	*139.5*	*0*	*139.5*	*104.6*	*104.6*	*69.7*
1/20/125 × Superlative; (PpTt mmFf) × (PpTt MmFf)	13	12+3	2	6	6+2	2	1	1	3	20	19+5	11	45	17	5
Expected	*12.2*	*12.2*	*4.1*	*4.1*	*4.1*	*1.4*	*5.4*	*5.4*	*1.8*	*21.7*	*21.7*	*7.2*	*69.7*	*10.8*	*14.5*

TABLE VII.

Parents	Red Hairy ♀	Red Hairy ♂	Red Glab ♀	Red Glab ♂	Tinged Hairy ♀	Tinged Hairy ♂	Tinged Glab ♀	Tinged Glab ♂	Green Hairy ♀	Green Hairy ♂	Green Glab ♀	Green Glab ♂	Total Sexes ♀	Total Sexes ♂	Total spine colour Red	Tinged	Green	Total hairiness Hairy	Glabrous
Norwich Wonder (selfed) (PpTt MMFf Hh)	53	11	24	8	21	7	9	0	13	2	8	2	128	30	96	37	25	107	51
Expected	*50.0*	*16.6*	*16.6*	*5.5*	*16.6*	*5.5*	*5.5*	*1.8*	*22.2*	*7.4*	*7.4*	*2.4*	*118.5*	*39.5*	*88.9*	*29.6*	*39.5*	*118.5*	*39.5*
Norwich Wonder Rogue × Norwich Wonder (P-TT M-FF Hh) × (PpTt MMFf Hh)	33	—	14	—	13	—	8	—	—	—	—	—	68	—	47	21	—	46	22
Expected	*38.2*	—	*12.7*	—	*12.7*	—	*4.2*	—	—	—	—	—	*68.0*	—	*51.0*	*17.0*	—	*51.0*	*17.0*
Norwich Wonder × Norwich Wonder Rogue (P-TT M-FF Hh) × (P-TT M-FF Hh)	51	—	17	—	14	—	5	—	—	—	—	—	87	—	68	19	—	65	22
Expected	*48.2*	—	*16.1*	—	*16.1*	—	*5.4*	—	—	—	—	—	*87*	—	*65.2*	*21.8*	—	*65.2*	*21.8*
Totals: Observed	84	—	31	—	27	—	13	—	—	—	—	—	155	—	115	40	—	111	44
Expected	*87.1*	—	*29.0*	—	*29.0*	—	*9.7*	—	—	—	—	—	*155*	—	*116.25*	*38.75*	—	*116.2*	*38.75*
Norwich Wonder × 7/20/6-47 (PpTt MMFf Hh) × (Pptt MMff Hh)	5	9	2	3	—	—	1	—	1	—	2	4	19+5	—	19	1	7	15	12
Expected	*3.8*	*3.8*	*3.8*	*1.3*	*1.3*	*1.3*	*0.4*	—	*5.0*	—	*1.7*	*1.7*	*5.0*	—	*10.1*	*3.4*	*13.5*	*20.2*	*6.75*

* 19 + 5 = 19 females + 5 weak females, see text.

selfed, and **Hh** × **Hh**, a larger proportion of sub-glabrous forms has appeared than normally expected.

TABLE VIII.

Parents	Observed		Expectation on a 3 : 1 basis	
	Hairy	Sub-glabrous	Hairy	Sub-glabrous
Hh selfed	107	51	118·5	39·5
Hh × **Hh**	146	76	166·5	55·5
hh selfed	0	665	0	665
hh × **hh**	0	822	0	822

So far we have not found any homozygous **HH** forms and it will be noted that the total results of heterozygous **Hh** forms both selfed and intercrossed approximate more closely to a 2 : 1 than a 3 : 1 ratio. This suggests that the homozygous **HH** forms are suppressed by a lethal, but the proportion of sub-glabrous forms obtained in individual families has varied, and pending further work this suggestion is only advanced tentatively.

DISCUSSION.

Although the hermaphrodites when selfed or crossed and the female forms when crossed usually set fruit abundantly, a high degree of sterility occurs in raspberries. The percentage of inviable seed, though variable, has been considerable in almost every self and cross made. The variation in viability has ranged from 89 per cent. to 7 per cent. A total of 7135 seeds has given only 2079 seedlings (29 per cent.) and many of these failed to reach maturity. In several families a number of albinotic plants appeared. Most of these died shortly after germination, but a few survived for a considerable time.

The following figures indicate that the inviability is due to lethal factors. Ten fruits obtained from selfing Pyne's Royal gave 61 plants, or 6·1 plants per fruit. Ten fruits from Lloyd George selfed gave 96 plants or 9·6 plants per fruit. But two fruits from Lloyd George × Pyne's Royal gave 88 plants or 44 per fruit. The greatest inviability from crossing was found in a cross between two sister seedlings (family 4/29), only 14 plants being obtained from each fruit produced. The viability of seeds from self-pollinations ranged from less than 2 to 32 plants per fruit. It therefore appears probable that lethal factors in the homozygous condition eliminate many of the zygotes, and if these factors are in any way involved with those producing spine colour then aberrant ratios will occur in certain crossed and selfed families. There is no close linkage between any factors, though **P** and **F** show a suspicion of slight linkage in two families.

SUMMARY.

In the raspberry, *Rubus idaeus* L., four sex forms occur, hermaphrodite, female, male and neuter. These forms are the expression of genetic differentiation, and from crosses between them a close approximation to the expected Mendelian segregation is obtained. Two factors are concerned with sex: **FM** is ☿; **F** is ♀; **M** is ♂ and **mf** is neuter. The male condition is correlated with obtuse foliage and approximates to *R. idaeus obtusifolius* Willd. The segregation with respect to the suppression of ♂ organs is not so sharply discontinuous as that of the ♀ organs.

The colour of the spines is correlated with the colour of the fruits. Red- and tinged-spined forms have red fruits, green-spined forms yellow fruits. **PT** gives red spines and fruits; **T** tinged spines and red fruits, **Ptt** and **ptt** green spines and yellow fruits. **P** intensifies the colour. The spine colour of **P** and **pt** is green, but it is probable that the colour of the fruits of **Ptt** is distinguishable from **pptt**, those of **Ptt** having a very faint tinge of red when fully matured, whilst those of **pptt** are a clear yellow.

Hairiness is dominant to the sub-glabrous condition. **H** = hairy growth, **h** = sub-glabrous.

Genetic differentiation of sex in *R. idaeus* is probably homologous with the basis of the sexual dimorphism found in *R. chamaemorus*. It is suggested that this condition is one from which alternative chromosome types might originate as the result, not the cause, of sexual differentiation.

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PLATE VII
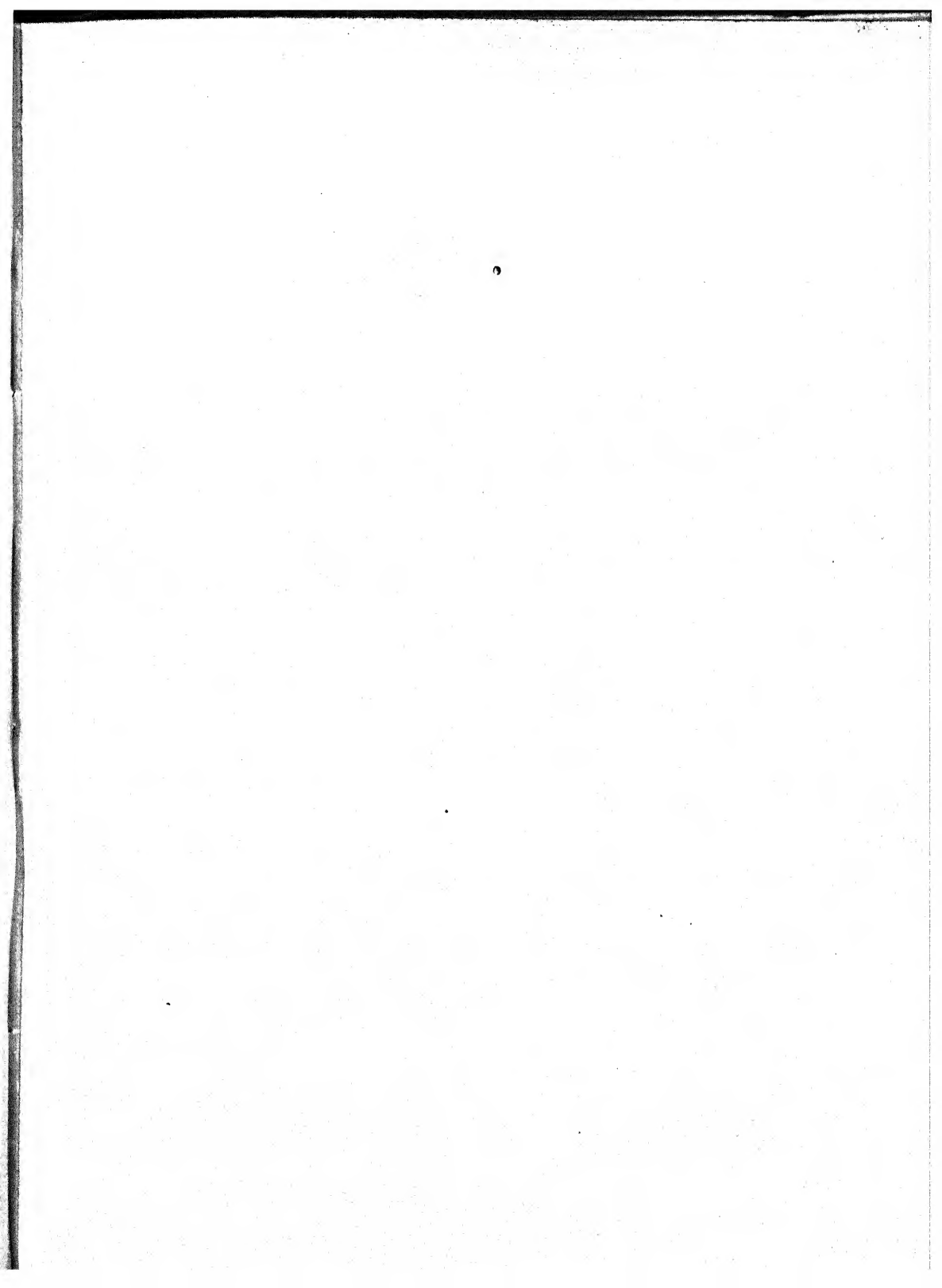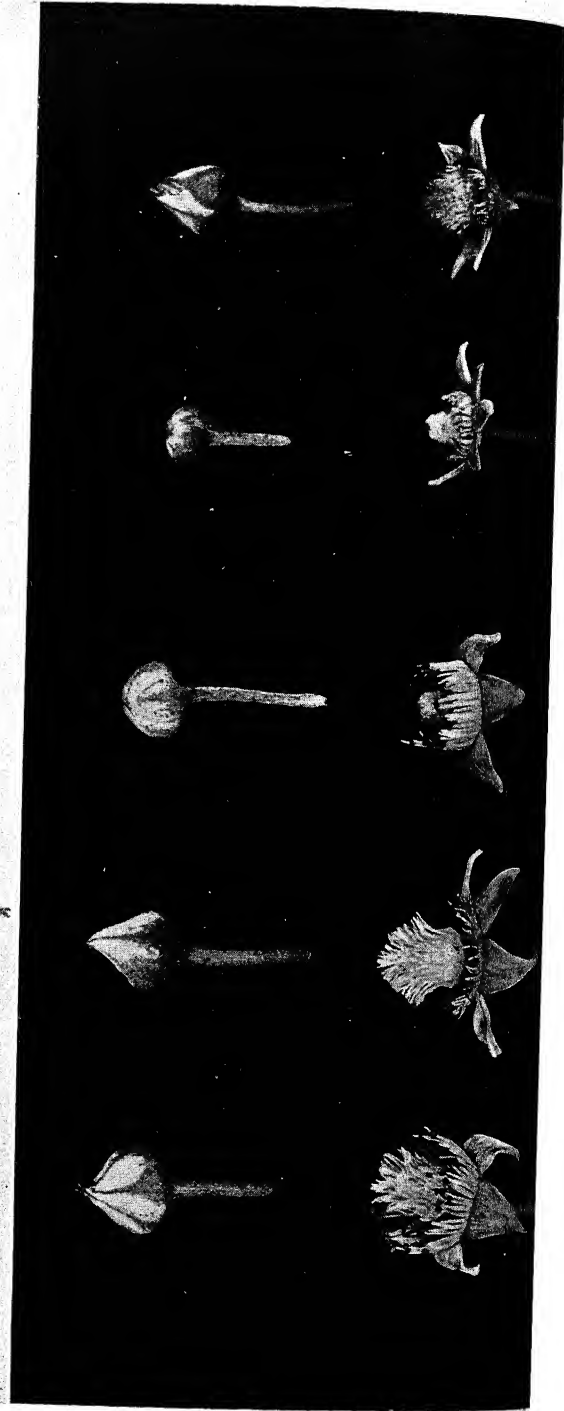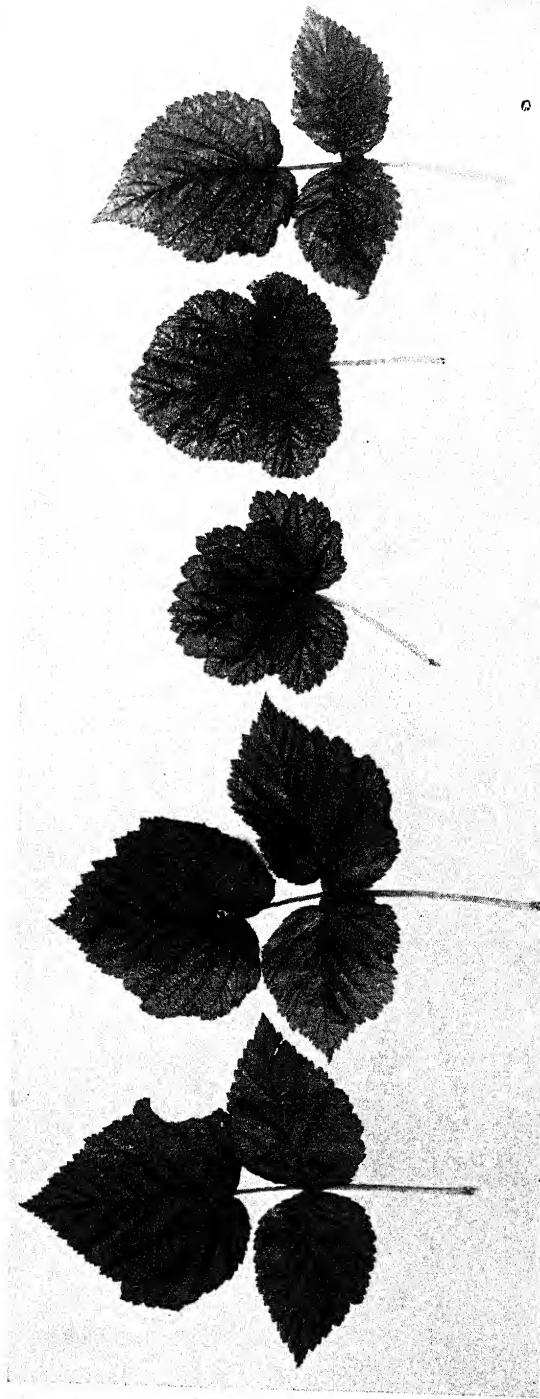
Fig. 1.

Fig. 3.
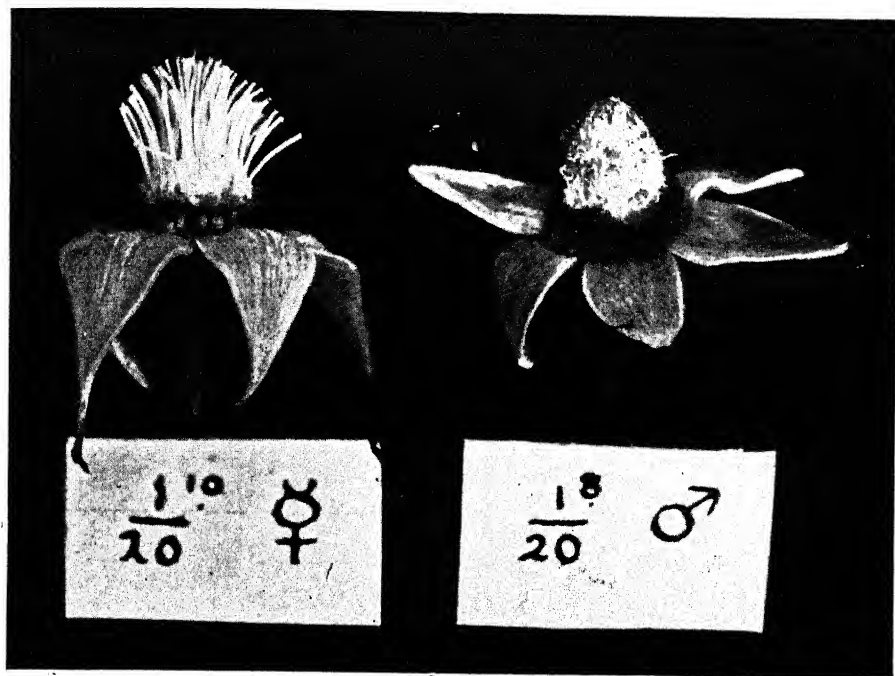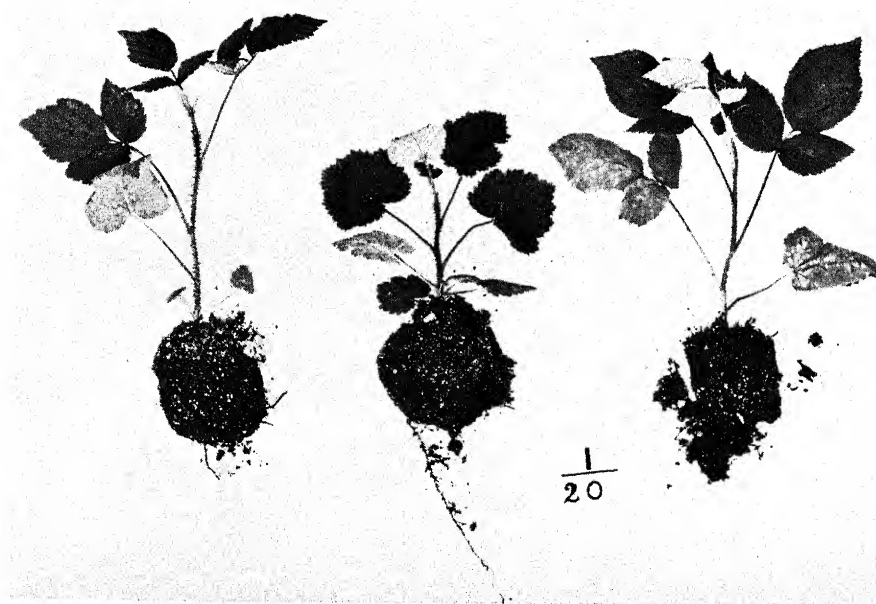
Fig. 2.

Fig. 4.
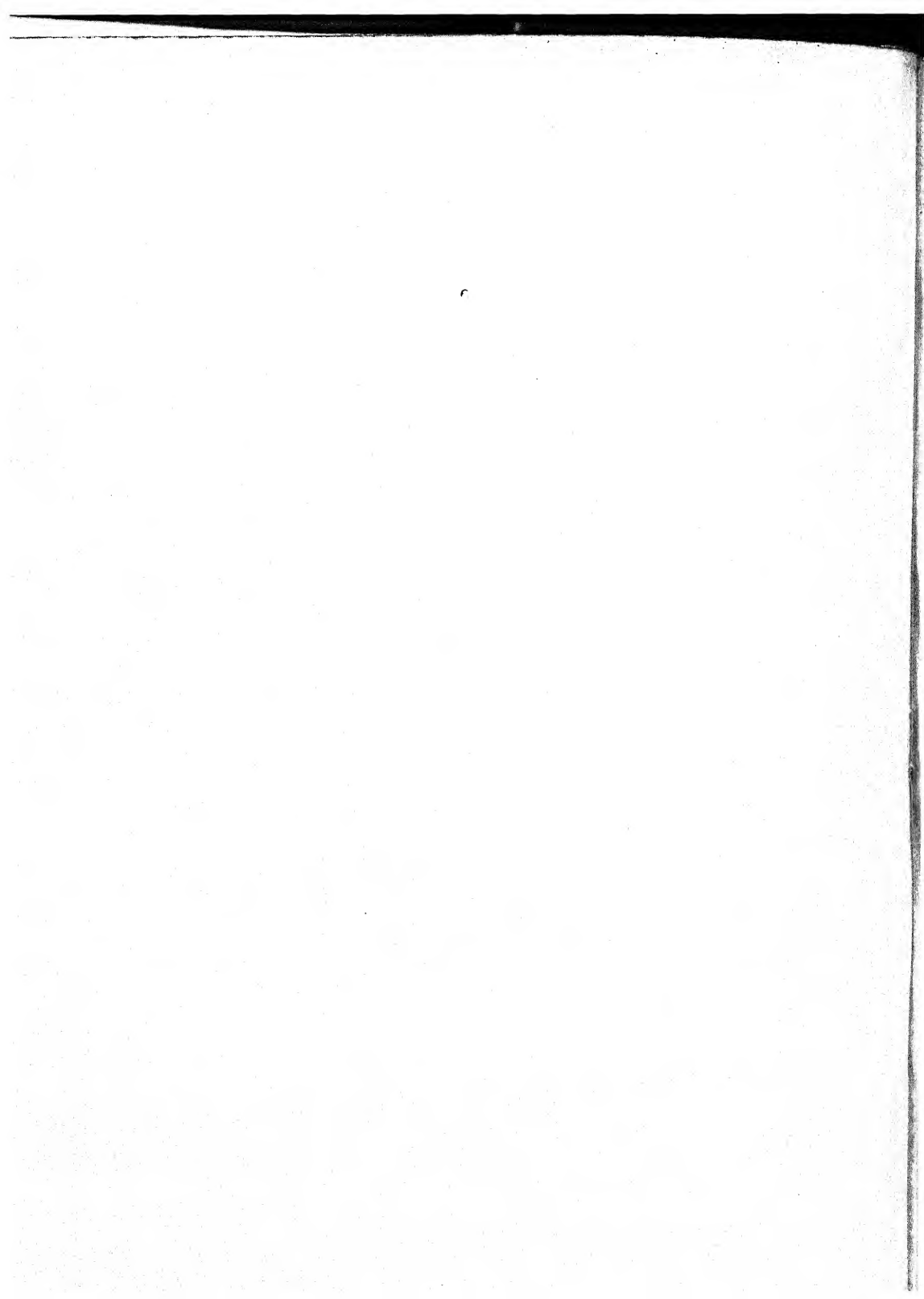
DESCRIPTION OF PLATES VII AND VIII.

Fig. 1. Flowers, flower buds and leaves of hermaphrodite (A), female (B), male (C), neuter (D) and weak female (E) raspberries. The leaves are from flowering canes, *i.e.* two-year-old growth.

Fig. 2. Enlarged photograph of hermaphrodite and male flowers. The flowers have been emasculated.

Fig. 3. Growth of Superlative (A) and selfed derivatives of Superlative (B, C, D). A and B are hermaphrodite, C female and D male. This, the first year's growth, is often referred to as the "sterile" growth, as normally flowers are not produced until the second year.

Fig. 4. Plants of family 1/20 in the seedling stage. B is a male plant and A and C either hermaphrodite or female.
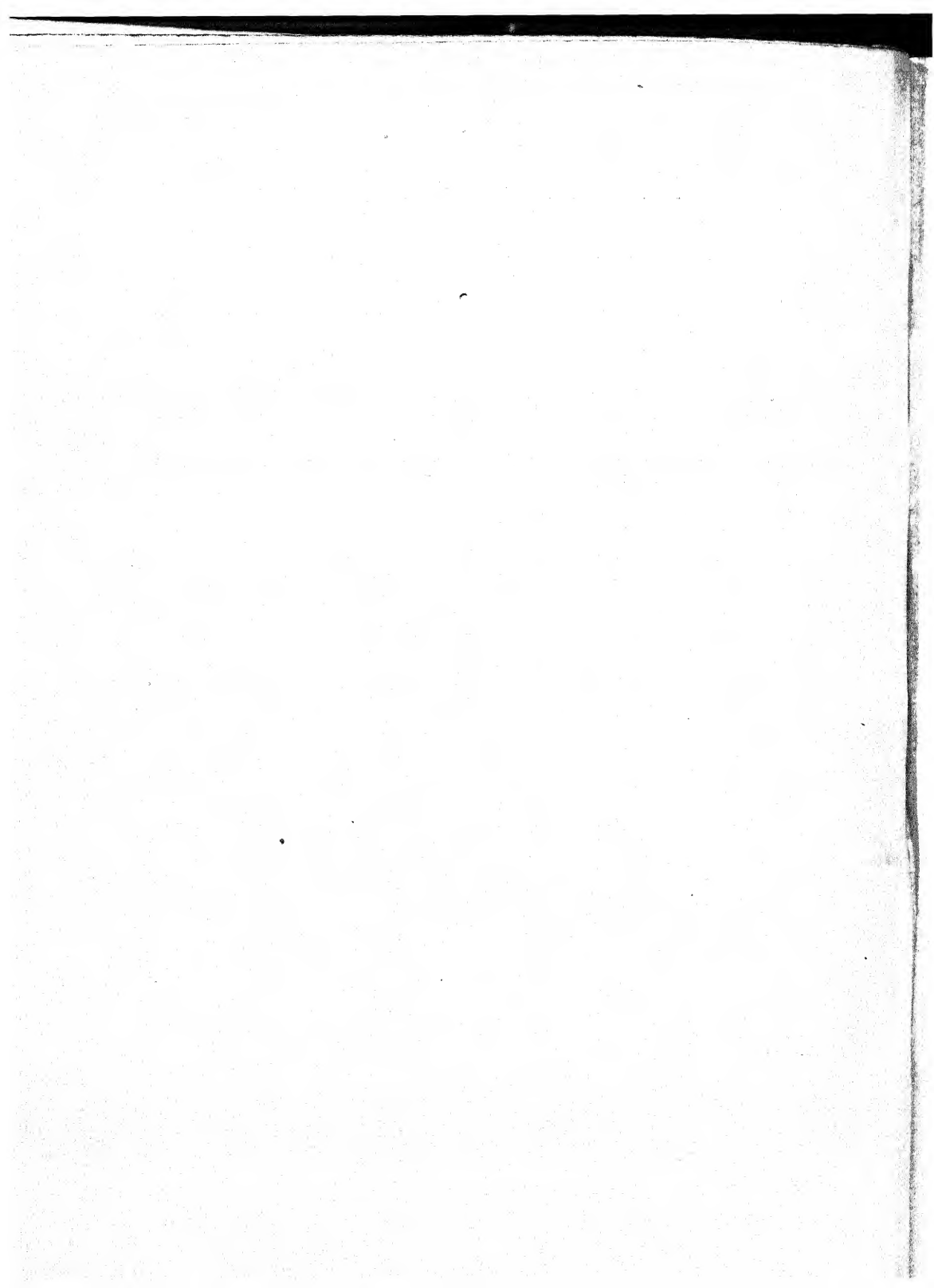
THE GENETICS AND CYTOLOGY OF *DAHLIA VARIABILIS*.

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(With Plate IX and Eight Text-figures.)

CONTENTS.

INTRODUCTION.

IN a preliminary account of the genetics and cytology of *Dahlia* (Lawrence, 1929) it was shown that, with the exception of *D. variabilis*, *Dahlia* species could be divided into two distinct groups for flower colour: Group I (ivory-magenta)[1] and Group II (yellow-orange-scarlet). Both series occur within *D. variabilis*. Flower colour in this species is the expression of two series of soluble pigments: (a) the flavones, (b) the anthocyanins. The flavones constitute the "ground" colours upon which the anthocyanins are superposed. The ground colours range from ivory to deep yellow. The anthocyanin colours appear as magenta to purple, or orange to scarlet, according to the intensity of pigmentation and the colour of the ground upon which they are superposed. Ivory florets can be recognised from white by fuming them with ammonia. White gives no reaction; ivory turns a good lemon colour; yellow changes to an intense orange. All ivories and whites in these experiments have been tested by fuming.

[1] The original colour range given was ivory-magenta-purple, but further work shows that purple should probably not be included.

D. variabilis is a self-incompatible octoploid with 64 chromosomes.

The evidence of the preliminary work suggested that *D. variabilis* was the derivative, by doubling of the chromosome complement, of a sterile hybrid between two tetraploid species, one belonging to the ivory-magenta and the other to the yellow-orange-scarlet colour group.

BREEDING RESULTS.

(a) *Flavone colours.*

Breeding experiments have shown that yellow flower colour is determined by the factor **Y** which is tetrasomic and segregated at random. Ivory flower colour is governed by the factor **I** which gives disomic ratios only. **yi** is white; **yI** ivory; **Yi** and **YI** yellow, the ivory colour being completely masked by the presence of yellow.

Table I presents the results obtained in 1929–30. Several of the families are reciprocal crosses, but since no significant difference has been found between these reciprocals they have been grouped together to avoid excessive detail.

Family 41/28* has been included, as 41/29 is a repetition of this cross. The total numbers are in close agreement with expectation.

The pom-pom variety Ideal had previously been assumed to be simplex for **Y** and recessive or heterozygous for **I**. In families 7/30, 8/30 and 13/30 Ideal has been crossed to **yyyy** seedlings, giving a total of 14 yellow to 14 non-yellow individuals. These figures strongly support the assumption that Ideal is simplex for **Y**. These same three families also show that it is probably heterozygous for **I**.

The parents of family 35–37/30 are sister seedlings from 32/26 (**yyyyii**) × 31⁵/27 (**Yyyyii**) and must therefore be simplex for **Y**. Either 41¹/28 or 41²/28 is heterozygous for **I**, the other being recessive, as the ratio 42 yellow : 5 ivory : 8 white is a close approximation to expectation from **YyyyIi** × **Yyyyii**. The ratio of 4 ivories to 1 white obtained in families 33 and 34/30 points to 41¹/28 as being heterozygous for **I**.

Families 14/30, 15/30, 29/30, 37/30 and 44/30 give results which confirm the constitutions previously assumed for their parents. The occurrence of 1 white individual in 15/30 is discussed later.

With the exception of certain families of which 2³/28 is one parent, the segregation of **Y** is usually without complication, and reference to the table will show the results obtained and expected. The segregation

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* In this and following tables the figures from one or two families previously reported upon have been included in the gross totals.

TABLE I.

Family	Parents	Observed Yellow	Ivory	White	Expected Yellow	Ivory	White
41/28, 41/29	32/26 (**yyyyii**) × 31⁵/27 (**Yyyyli**)	28	12	15	*27·5*	*13·8*	*13·8*
28/30	32/26 (**yyyyii**) × 6²/29 (**Yyyy(ii?)**)	4	0	3	*3·5*	*0*	*3·5*
7/30, 8/30	White Star (**yyyyIi**) × Ideal (**YyyyIi**)	10	9	1	*10·0*	*7·5*	*2·5*
13/30	Ideal (**YyyyIi**) × 32/26 (**yyyyii**)	4	1	3	*4·0*	*2·0*	*2·0*
11/30, 12/30	2²/28 (**YyyyIi**) × 32/26 (**yyyyii**)	23	8	8	*19·5*	*9·8*	*9·8*
16/30	35/26 (**yyyyii**) × 2²/28 (**YyyyIi**)	20	11	6	*18·5*	*9·3*	*9·3*
17/30	2³/28 (**YYyyII**) × 35/26 (**yyyyii**)	32	8	4	*36·7*	*7·3*	*0*
23/30	Everest (**yyyyII**) × 2²/28 (**YyyyIi**)	15	22	0	*18·5*	*18·5*	*0*
24/30, 25/30	2²/28 (**YyyyIi**) × 10/29 (**YyyyII**)	34	34	0	*34·0*	*34·0*	*0*
14/30	34/26 (**YYyyIi**) × White Star (**yyyyIi**)	22	5	0	*22·5*	*4·5*	*0*
15/30	34/26 (**YYyyII**) × Everest (**yyyyII**)	11	3	1	*12·5*	*2·5*	*0*
37/29	22⁹/27 (**YYyyIi**) × 35/26 (**yyyyii**)	21	2	1	*20·0*	*2·0*	*2·0*
9/30, 10/30	2³/28 (**YYyyII**) × 32/26 (**yyyyii**)	38	6	2	*38·3*	*7·7*	*0*
22/30	2³/28 (**YYyyII**) × Everest (**yyyyII**)	35	10	1	*38·3*	*7·7*	*0*
20/30, 21/30	10¹/28 (**yyyyIi**) × 2³/28 (**YYyyII**)	68	12	4	*70·0*	*14·0*	*0*
18/30	2³/28 (**YYyyII**) × White Star (**yyyyIi**)	21	8	0	*24·0*	*4·8*	*0*
6/29, 35–37/30	41¹/28 (**YyyyIi**) × 41²/28 (**Yyyyii**)	42	5	8	*41·3*	*6·9*	*6·9*
33/30, 34/30	41¹/28 (**YyyyIi**) × 2²/28 (**Yyyyli**)	14	4	1	*14·2*	*3·6*	*1·2*
55/30	31⁵/27 (**YyyyIi**) × 2²/28 (**YyyyIi**)	38	12	0	*37·5*	*9·4*	*3·1*
40/30, 41/30	2³/28 (**YYyyII**) × 41¹/28 (**YyyyIi**)	83	9	0	*84·2*	*7·7*	*0*
54/30	2³/28 (**YyyyIi**) × 31⁵/27 (**YyyyIi**)	72	8	0	*73·3*	*6·7*	*0*
29/30	32/26 (**yyyyii**) × 14/26 (**YYYyIi**)	69	0	0	*69·0*	*0*	*0*
44/30	14/26 (**YYYyIi**) Selfed	1	0	0	*1·0*	*0*	*0*

TABLE II.

Family	Parents	Ground colours Observed Yellow	Ivory	Expected Yellow	Ivory
56–59/30	Glenshee (apricot) (**YyyyII**) × White Star (ivory) (**yyyyIi**)	·93	86	*89·5*	*89·5*
31/30	Coltness Gem (scarlet) (**Yyyy––**) × Everest (ivory) (**yyyyII**)	15	16	*15·5*	*15·5*
61/30	32/26 (white) (**yyyyii**) × Union Jack (scarlet) (**YyyyII**)	32	21	*26·5*	*26·5*
22/27, 29–35/29	Union Jack (scarlet) (**YyyyII**) × Glenshee (apricot) (**YyyyII**)	274	79	*264·8*	*88·3*
12/29	36/26 (magenta) (**yyyyII**) × M 5 (scarlet) (**YYyyI–**)	33	7	*33·3*	*6·7*
45/30	White Star (ivory) (**yyyyIi**) × M 5 (scarlet) (**YYyyI–**)	12	5	*14·2*	*2·8*
26/27, 31/27, 39/29, 40/29, 62/30	Union Jack (scarlet) (**YyyyII**) × 34/26 (yellow) (**YYyyII**)	191	14	*187·9*	*17·1*
60/30	14/26 (yellow) (**YYYyIi**) × 1²/25 (scarlet) (**YYyyII**)	69	0	*69·0*	*0*
7/29, 46–48/30	14/26 (yellow) (**YYYyIi**) × M 5 (scarlet) (**YYyy––**)	189	0	*189·0*	*0*
50–52/30	14/26 (yellow) (**YYYyIi**) × M 8 (purple) (**yyyyII**)	152	1*	*153·0*	*0*
3/29	15¹/28 (scarlet) (**Y––––**) × 14/26 (yellow) (**YYYyIi**)	59	2*	*61·0*	*0*
42/29	14/26 (yellow) (**YYYyIi**) × 22⁸/27 (orange) (**Y–yyII**)	22	0	*22·0*	*0*
16–18/29, 21/29	14/26 (yellow) (**YYYyIi**) × 27/27 (yellow grounds)	92	0	*92·0*	*0*

of **I**, however, is often anomalous. The discussion of these unexpected results is left until later in the paper, since other data are necessary to the discussion.

Table II shows the inheritance of **Y** in crosses between parents with yellow or ivory *grounds* (*i.e.* with anthocyanin present). Glenshee had previously been assumed to be duplex for **Y** from its behaviour in a cross with Union Jack, but it is now clear from the larger families raised that Glenshee is simplex for **Y**. The unexpected recessives in families 50–52/30 and 3/29 are probably the result of double reduction (see Lawrence 1929, p. 138). With the exception of the 1927 families, all individuals whose ground colour was uncertain were tested by bleaching out the anthocyanin with sulphur dioxide. The scoring of yellow and ivory grounds in the crosses between "coloured" plants can therefore be regarded as critical.

TABLE III.

Genotypes	Observed		Expected		Goodness of fit
	Y	**y**	*Y*	*y*	*P*
Yyyy × yyyy	303	281	*292·0*	*292·0*	·38
Yyyy × Yyyy	375	110	*363·8*	*121·3*	·17
YYyy × yyyy	399	95	*411·7*	*82·3*	·13
YYyy × Yyyy	480	45	*481·3*	*43·8*	0·8
YYYy × yyyy	378	7	*385·0*	*0*	—
YYYy × Yyyy	223	1?	*224·0*	*0*	—
YYYy × YYyy	258	0	*258*	*0*	—

The inheritance of **Y** is summarised in Table III. As shown in Table I, 12 white-rayed seedlings appeared in certain crosses where none was expected. These whites are all in the class **YYyy × yyyy**. As will be shown later, there is little doubt that these whites are due to factors which inhibit the formation of the flavone colours. The twelve whites therefore should be added to the yellow, or yellow and ivory classes, which then closely approximate to the expected numbers (e.g. 411 **Y** : 83 **y**). The seven non-yellow individuals in class **YYYy × yyyy** are likewise due to inhibitors, or double reduction.

Only two small families have been raised further from plants with ivory or white flowers. In both cases Everest was one of the parents.

TABLE IV.

		Ground colours			
		Observed		Expected	
Family	Parents	Ivory	White	Ivory	White
5/30	Everest (ivory) **II** × 32/26 (white) **ii**	9	3	*12*	*0*
6/30	Everest (ivory) **II** × White Star (ivory) **Ii**	10		*10*	*0*

The variety Everest has ivory ray-florets. In many cases it is possible to distinguish plants which are heterozygous for **I** from those which are homozygous, for the action of **I**, unlike that of **Y**, appears to be cumulative*. Everest has deep ivory flowers and should therefore be homozygous for **I** (cf. Table I), but when crossed with 32/26 (family 5/30) 3 whites appeared in a total of 12 individuals. Because these white individuals were not expected, they were tested by fuming many times during the summer in order to be certain of their nature. Finally, one plant which had hitherto borne white flowers produced on different capitula several ivory sectors and petals. The distribution of the ivory pigment was sharply defined, and included whole petals or large sectors. Although many capitula were tested on the other three seedlings, no trace of ivory was found. The same phenomenon had been encountered in the individual 35/26 raised from seed from a nurseryman. 35/26 is almost always white, but occasionally produces ivory sectors and petals.

It seems probable that an inhibitor of ivory totally suppresses that pigment in the case of the 5/30 seedlings and 35/26, and that loss mutation of the inhibitor allows the pigment to develop in the affected areas. Sectorial mutation of ivory, yellow and the anthocyanin colours is relatively common in *D. variabilis*; and I have observed and collated many hundreds of such mutations, since they are of considerable value in indicating and confirming the constitution of plants for flower colour. Six or seven cases similar to the Everest seedling, which later produced ivory sectors, were recorded in other families in 1930.

The yellow pigment may be inhibited in a similar way. Two such examples appeared in families 20 and 21/30 (10¹/28, ivory edges × 2³/28, primrose). 10¹/28 is a seedling from 32/26 (**yyyyii**) × White Star (**yyyyIi**), and has the ivory pigment only in the edges of the petals. 2³/28 is from 14/26 (**YYYyIi**) × White Star. The types of colour pattern and segregates found in 20 and 21/30 have not been seen previously in these experiments, which would seem to indicate that the incidence of certain factors in a relatively homozygous and probably recessive condition is permitting the expression of new characteristics. It will be noted that 10¹/28 and 2³/28 are closely related, and that families 20 and 21/30 result from inbreeding. Two seedlings in 21/30, a cream and a primrose,

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* "Blended inheritance" and "incomplete dominance" result from the cumulative effect of like factors. In polyploids, as in the comparable condition in diploids, the saturation point may be reached at once in the simplex form. This is complete dominance. On the other hand, it may lie at or beyond the point where *all* the like factors exert their maximum effect. In this case the addition of each factor necessarily increases the total

gave ivory sectors rather frequently, and in the case of the cream seedling whole ivory petals occurred. The ivory in the cream seedling is sometimes only in the edges.

A striking example of the inhibition of yellow was found in family 31/30 (Coltness Gem × Everest). Two seedlings with magenta flowers produced apricot sectors, and one of these seedlings an entire apricot petal, *i.e.* the ivory ground had changed to yellow. The reverse mutation is commonly found, but this was the first time the change from magenta to apricot had been seen. Further, in the same family two purplish-crimson seedlings sported crimson sectors, *i.e.* the ivory ground changed to yellow. The reverse mutations—apricot to magenta and crimson to purplish-crimson (see Plate IX, fig. 11)—occurred several times in 31/30, and thus the unexpected and usual colour changes could be compared and were found to be reciprocally identical. It will be noted that Everest is one of the parents of 31/30.

In 1929 (p. 141) I reported that one cross (2/28 × 3/28) gave colours intermediate between ivory and yellow. The parents—14/26 and White Star—of these two families had always given yellows, ivories or whites in other crosses, but in 2/28 and 3/28 creams and primroses occurred, and other forms with the pigment fading from the base to the tip of the petals, *e.g.* yellow to cream, primrose to ivory, etc. Five ivories also occurred when none was expected, for 14/26 is triplex for **Y** and, meiotic irregularities excepted, cannot give non-yellow gametes. The scoring of the intermediate grades was difficult, but a total of 14 creams, 51 primroses and 14 yellows was obtained. It was shown that interaction between **I** and **Y** did not account for these intermediates, and it was suggested that a tetrasomic inhibiting factor with a cumulative effect upon the yellow flavone might be responsible, since the ratio 14 : 51 : 14 is close to 1 : 4 : 1, *i.e.* the expectation when a plant duplex for an inhibitor is crossed to the recessive.

Seedlings $2^2/28$ and $2^3/28$ were cream and primrose respectively, selected for further testing of the postulated inhibiting factor. Numerous attempts were made to cross these seedlings with their male parent, White Star, and with each other, but no seed was obtained. These three plants are apparently cross-incompatible.

In every cross made with $2^2/28$ or $2^3/28$ a large number of cream and primrose forms has occurred, and, especially in families 20 and 21/30, the suppression of yellow is extreme. In several cases the yellow pigment has been so dilute as practically to defy detection in fully expanded capitula, and only examination in the bud stage has shown the yellow

to be present. Similarly the ivory pigment has also been found to vary in degree, the reaction with ammonia being almost nil in some cases.

Referring back to Table I, we are now in a position to examine the inheritance of ivory in these families. The results obtained when $2^3/28$ is used as parent are rather indefinite. Crossed with forms lacking **Y** it gives 160**Y** : 45**y**, which is closer to a 3 : 1 than a 5 : 1 ratio. Yet when $2^3/28$ was crossed with two **Yyyy** seedlings it gave 155**Y** : 17**y**, which is close to expectation (157·6**Y** : 14·3**y**) on the assumption that $2^3/28$ is duplex for **Y**. It is probable that such is the constitution of this seedling, for the deficiency of yellows in crosses with nulliplex forms is mainly explained by the occurrence of white varieties where none is expected. Crossed with White Star (**yyyyIi**), $31^5/27$ and $41^1/28$ (both **YyyyIi**), $2^3/28$ gave no whites in a total of 201 plants, thereby indicating that it is homozygous for **I**. Had it been heterozygous 50 whites would have been expected.

In family 9/30, however, 2 whites occurred: 4 in 17/30 and 4 in 20 and 21/30, all crosses with the bottom recessive types. Finally 1 white was found in each of families 15/30 and 22/30. In these last families there are too many ivories, and along with the other crosses just cited it is apparent that inhibition of yellow and ivory accounts for the disturbed ratios and anomalous whites. $2^2/28$ is apparently heterozygous for **I**, since a total of 25 ivories to 20 whites was obtained from crosses with recessive forms.

The argument that the unexpected whites result from selfing is untenable, for they have appeared most often when the female parent is of such a constitution as virtually to preclude this possibility. The question of selfed seedlings arising from cross-pollinations is dealt with later.

The disomic character of the factor **I** is confirmed by these later experiments. A total of 23 distinct individuals has now been analysed for their constitution with respect to **I**. 13 were **II**, 7 **Ii** and 3 **ii**.

(b) *Flavone inhibitors.*

As already stated, the first evidence of the action of a cumulative inhibitor of yellow came from the family 14/26 (yellow) × White Star (ivory) which gave 14 yellow : 51 primrose : 14 cream individuals. It seems probable that a distributing factor complicates the situation with regard to the inhibitor, for self-coloured yellows, primroses and creams occur along with forms in which yellow, primrose or cream shade off to a paler colour towards the tips of the rays. The presence of a yellow eye of varying size adds further to the difficulty of scoring.

It is noteworthy that family 29/30 (32/26 × 14/26) again gave a 5 : 1 ratio, *i.e.* 56 yellow : 10 primrose. No cream forms occurred in this cross, however, and the yellows could be further divided into 12 deep, 26 medium and 18 pale—the last class corresponding to the yellow of 14/26.

Families 20 and 21/30 (10¹/28 (ivory) × 2³/28 (primrose)) gave 11 primrose : 53 cream individuals, the creams varying from deep to very pale cream. This again is close to a 5 : 1 ratio. 2³/28 crossed by 35/26 (white) and White Star (ivory) gave 17 primrose : 15 cream, and 11 primrose : 10 cream respectively. 2³/28 was also crossed with 31⁵/27 (yellow) and gave 23 yellow : 44 primrose : 1 cream. 2²/28 × 31⁵/27 gave 15 yellow : 17 primrose : 2 cream.

On a tetrasomic basis the 5 : 1 ratio can only be given by a factor in the duplex condition. Three such ratios have appeared in the inheritance of the yellow inhibitor, but in each case the phenotypes were different. This probably indicates interaction of the inhibitor with some other factors—probably the colour factors. Moreover, it is not improbable that a second inhibitor (cf. the flavone and anthocyanin factors) functions somewhat differently from the first. "Presence" or "absence" of this second factor might definitely shift the colour grade as determined by the first inhibitor. The evidence relating to the inheritance of the yellow inhibitor(s) is at present indefinite, but the gross results from several families indicate that one or more tetrasomic factors are concerned.

(c) *Anthocyanin colours.*

A study of the inheritance of anthocyanin in *D. variabilis* was at first thought to be impracticable, (1) owing to the complexity of the colours, and (2) because until 1929 all of the families raised were grown for the study of a peculiar flower-colour pattern described in the paper accompanying this account. The parents used were therefore chosen indiscriminately as far as colour was concerned, but a fortunate combination of circumstances revealed the basis of anthocyanin inheritance.

The first indication that there was more than one factor for anthocyanin production came from some pale-coloured seedlings out of a cross between two individuals with rather deep-coloured flowers. These light-coloured seedlings (cf. Plate IX, figs. 1–6) were crossed together but never gave deeper-coloured offspring, an observation which has been confirmed by further work.

The factorial constitution of *D. variabilis* for flower colour was ultimately revealed by a large family from reciprocal crosses between Glenshee and Union Jack. Glenshee has apricot flowers typical of the

light-coloured seedlings mentioned, whereas Union Jack has intense crimson-scarlet flowers. The progeny from this cross came in the proportion of 7 with anthocyanin to 1 without, which is expectation when the parents carrying two independent, tetrasomic factors are **Aaaabbbb** and **AaaaBbbb** respectively.

The results from Glenshee × Union Jack are particularly clear for the following reasons. Both are homozygous for **I** and all the progeny have a uniform ivory ground—with or without yellow. Union Jack is simplex for three factors, Glenshee simplex for two and nulliplex for the third: hence the ratio of dominants to recessives in F_1 never exceeds 3 : 1, and the number of each class obtained is sufficient to give a reliable measure of class frequency. In addition, the yellow grounds are of uniform intensity, the yellow-inhibiting factor not expressing itself, so that a direct comparison of the anthocyanin colour-differences is possible.

In most of the tables showing anthocyanin inheritance, the total figures are derived from several reciprocal crosses. No significant difference has been found in reciprocal crosses, which are therefore grouped together to avoid excessive detail.

As shown in Table V, the colours grade very finely from apricot to magenta. From apricot to scarlet inclusive the differences are purely quantitative, *i.e.* differences of intensity. Similarly from ivory to purple the differences are apparently differences of degree. It is impossible to say from observation whether the differences between the very intense middle colours are quantitative or qualitative. In all these experiments the colours have been matched with standard types, and only flowers of the same age used, since the dahlia fades considerably with age.

The interpretation of the factorial scheme for anthocyanin flower colour is as follows. **A** produces a relatively pale coloration and is cumulative in its effect. **B** produces deeper pigmentation than **A** and is almost entirely cumulative, but pending further work this is not certain. Thus, **A** simplex plus ivory gives pale magenta, but with yellow the colour is apricot. **B** simplex with ivory is purple, with yellow, scarlet. In addition to the cumulative effect of **A** and (probably) **B**, modifying factors or conditions also influence the degree of pigmentation, and in the family Glenshee × White Star where all coloured progeny are **A**-simplex with or without **Y**, the magentas and apricots vary from faintly-tinged to deep magenta or apricot.

The grouping of the variates in Table V under the zygotic types as shown does not purport to be a correct assignment of the colour classes to their respective genotypes, but is simply the best approximation to

expectation which is obtained by taking the colour classes in successive order with due reference to the effect of **A** and **B**.

It is highly probable that overlapping of the phenotypes occurs in several of the genotypic classes—especially among the deep-coloured phenotypes. This seems to be especially true in the case of the phenotypes with ivory grounds, since no arrangement will give an even moderately good fit. The magenta class (**Aby**) is, in some families, easy to score and very distinct, but in all cases where Union Jack is one parent there is apparently a considerable deficiency of magentas, whilst rosy-purples

TABLE V.

Union Jack (crimson-scarlet) (**YyyyIIAaaaBbbb**) × *Glenshee (tinged apricot)* (**YyyyIIAaaabbbb**).

Ref. no.	Flower colour	Plants		Observed	*Expected*
I	Yellow	36			
II	Faintly tinged apricot	13			
III	Apricot	7			
IV	Orange	45			
V	Scarlet-orange ...	9			
VI	Orange-scarlet ...	24	Yellow grounds	274	*264·8*
VII	Scarlet	39			
VIII	Crimson-scarlet ...	68			
IX	Scarlet-crimson ...	28			
X	Crimson	5	No anthocyanin	44	*44·1*
XI	Purplish-crimson ...	7	Anthocyanin	309	*308·9*
XII	Crimson-purple ...	6			
XIII	Purple	14			
XIV	Rosy purple	34	Ivory grounds	79	*88·3*
XV	Magenta-purple ...	6			
XVI	Magenta	4			
XVII	Ivory	8			
		Total 353			

Expectation (see text)

Ref. no.	Zygotic types	*Expected*	Observed	Ref. no.	Zygotic types	*Expected*	Observed
I	**abY**	*33·1*	36	XI, XII	**A₂By**	*11·0*	13
II, III, IV	**AbY**	*66·2*	65		**ABy**	*22·1*	
V, VI	**A₂bY**	*33·1*	33	XIII–	**aBy**	*11·0*	
VII	**aBY**	*33·1*	39	XVI	**A₂by**	*11·0*	58
VIII	**ABY**	*66·2*	68		**Aby**	*22·1*	
IX, X	**A₂BY**	*33·1*	33	XVII	**aby**	*11·0*	8

are in excess. A possible explanation of this discrepancy is suggested by the mutations that occur among certain apricots in this and other families. If the yellow ground of an apricot flower mutates to ivory, then the colour in that sector appears magenta, but almost invariably the magenta is at least twice as deep as the adjacent apricot. This increase in intensity considered along with the deficiency of pale anthocyanin-coloured forms with ivory grounds suggests that the combined effect of **A** and **I** in certain families is different from that of **A** and **Y**. It is

probable that this is due to the action of modifying factors, since it is only found in particular crosses.

On the assumption that Glenshee is **YyyyIIAaaabbbb**, it should give equal numbers of ivory and yellow grounds and magentas and apricots when crossed to the triple recessive form. Table VI shows the actual results. It is noteworthy that the magenta class is again too small.

TABLE VI.

Glenshee (tinged apricot) (**YyyyIIAaaabbbb**) × *White Star (ivory)*
(**yyyyIiaaaabbbb**).

Flower-colour	Zygotic type	Expected	Observed	
Yellow	**abY**	*48·5*	49	Ob. 102**Y** : 92**y**
Apricot	**AbY**	*48·5*	53	Ex. 97 : 97
Magenta	**Aby**	*48·5*	37	Ob. 90**A** : 104**a**
Ivory	**aby**	*48·5*	55	Ex. 97 : 97

In the next cross—Table VII—the male parent 22⁸/27 is one of the F_1 seedlings from Union Jack × Glenshee. Crossed with 14/26 (**YYYyIiaaaabbbb**), 22⁸/27 gave 71 individuals with anthocyanin to 15 without, or almost exactly 5 : 1. Of the five possible genotypes for

TABLE VII.

14/26 *(yellow)* (**YYYyIiaaaabbbb**) × 22⁸/27 *(scarlet-orange)*
(**Y‑‑‑IIAAaabbbb**).

Ref. no.	Flower colour	Plants		Observed	Expected
I	Yellow	15			
II	Faintly tinged apricot	11			
III	Apricot	10	No anthocyanin	15	*14·3*
IV	Orange	27	Anthocyanin	71	*71·7*
V	Pale crimson-scarlet	2			
VI	Scarlet-orange	16			
VII	Scarlet	5			
	Total	86			

Expectation (see text)

Ref. no.	Zygotic type	Expected	Observed
I	**abY**	*14·3*	15
II, III, IV, V	**AbY**	*57·3*	50
VI, VII	**A₂bY**	*14·3*	21

22⁸/27 the only one which will give this ratio when crossed with a form lacking anthocyanin is **AAaabbbb**. This is the most probable constitution for a scarlet-orange individual. Although a satisfactory grouping of the colour classes in the F_1 of this family cannot be made, it is significant that 16 scarlet-orange individuals, identical with 22⁸/27, occur. Expectation is 14·3. If the tinged, apricot, orange and pale crimson-scarlet classes are summed, a total of 50 plants, probably simplex for **A**, is obtained. The scarlets are probably duplex for **A** but intensified

by subsidiary factors, in which case some of the scarlet-oranges may be simplex for **A**. The scarlets are being tested further.

The next three tables show the results from crossing Union Jack with different forms without anthocyanin. The expected ratio is 3 with anthocyanin : 1 without, and reference to Table VIII will show that this is realised in the cross 34/26 (yellow) × Union Jack. The faintly-tinged class does not appear in any of these three families, and other classes are not represented either. It is important to notice that the purplish-crimson and crimson-purple classes no longer have ivory grounds. This is because differences in the intensity of the yellow grounds definitely

TABLE VIII.

34/26 *(yellow)* (**YYyyIIaaaabbbb**) × *Union Jack (crimson-scarlet)*
(**YyyyIIAaaaBbbb**).

Ref. no.	Flower colour		Plants		Observed	*Expected*
I	Yellow	...	51			
II	Apricot	...	3			
III	Orange	...	21			
IV	Orange-scarlet		12			
V	Scarlet	...	6	Yellow grounds	191	*187·9*
VI	Crimson-scarlet		29			
VII	Scarlet-crimson		21			
VIII	Crimson	...	16			
IX	Purplish-crimson		17	No anthocyanin	52	*51·3*
X	Crimson-purple		15	Anthocyanin	153	*153·8*
XI	Purple	...	10			
XII	Magenta-purple		2	Ivory grounds	14	*17·1*
XIII	Magenta	...	1			
XIV	Ivory ...	...	1			
		Total	205			

Expectation (see text)

Ref. no.	Zygotic types	*Expected*	Observed	Ref. no.	Zygotic types	*Expected*	Observed
I	**abY**	*47·0*	51	XI	**ABy**	*4·3*	10
II, III, IV	**AbY**	*47·0*	36	XII	**aBy**	*4·3*	2
V, VI, VII	**aBY**	*47·0*	56	XIII	**Aby**	*4·3*	1
VIII, IX, X	**ABY**	*47·0*	48	XIV	**aby**	*4·3*	1

shift the phenotypic class to which a given genotype belongs. For example, on a good yellow ground a certain genotype may appear crimson, but if inhibitors suppress the formation of yellow so that a cream ground results then, since cream is very little different from ivory, the same genotype (as far as **I**, **Y**, **A** and **B** are concerned) may appear purplish—the slight difference between cream and ivory ground colours having practically no effect on the deep colour of the anthocyanin. Again there is a considerable overlapping of the classes with ivory grounds which cannot be satisfactorily classified. In the next family, Table IX, Union Jack was crossed with the bottom recessive form 32/26. The yellow grounds are fairly uniform and a fair approximation to $1Y : 1y$ is obtained. As in all

the other families the ivory and magenta classes are too small, with the result that the ratio of coloured to uncoloured is not significant.

TABLE IX.

32/26 *(white)* (**yyyyiiaaaabbbb**) × *Union Jack (crimson-scarlet)* (**YyyyIIAaaaBbbb**).

Ref. no.	Flower colour	Plants		Observed	*Expected*
I	Yellow	7			
II	Orange	5			
III	Scarlet-orange	3			
IV	Scarlet	2	Yellow grounds	33	*30·0*
V	Bleached scarlet-crimson	8			
VI	Scarlet-crimson ...	2	No anthocyanin	8	*15·0*
VII	Crimson	6	Anthocyanin	52	*45·0*
VIII	Purplish-crimson ...	1			
IX	Deep purplish-crimson	8			
X	Deep crimson-purple ...	5			
XI	Purple	8	Ivory grounds	27	*30·0*
XII	Purplish-magenta ...	2			
XIII	Deep magenta	2			
XIV	Ivory	1			
	Total	60			

Expectation (see text)

Ref. no.	Zygotic types	*Expected*	Observed	Ref. no.	Zygotic types	*Expected*	Observed
I	**abY**	*7·5*	7	VIII, IX, X	**ABy**	*7·5*	14
II, III	**AbY**	*7·5*	8	XI	**aBy**	*7·5*	8
IV, V	**aBY**	*7·5*	10	XII, XIII	**Aby**	*7·5*	4
VI, VII	**ABY**	*7·5*	8	XIV	**aby**	*7·5*	1

TABLE X.

Union Jack (crimson-scarlet) (**YyyyIIAaaaBbbb**) × 14/26 *(yellow)* (**YYYyIiaaaabbbb**).

Ref. nos.	Flower colour	Plants		Observed	*Expected*
I	Yellow	32			
II	Apricot ...	3			
III	Orange	30			
IV	Scarlet-orange...	13			
V	Orange-scarlet...	19		Observed	*Expected*
VI	Scarlet	20	No anthocyanin	32	*43·8*
VII	Crimson-scarlet	27	Anthocyanin	143	*131·3*
VIII	Scarlet-crimson	11			
IX	Crimson ...	12			
X	Purplish-crimson	7			
XI	Crimson-purple	1			
	Total	175			

Expectation (see text)

Ref. nos.	Zygotic types	*Expected*	Observed
I	**abY**	*43·8*	32
II, III, IV	**AbY**	*43·8*	46
V, VI	**aBY**	*43·8*	39
VII–XI	**ABY**	*43·8*	58

Table X shows the results from 14/26 (yellow) × Union Jack. A deficiency of yellows is found. No ivory grounds occur since 14/26 is

triplex for **Y**, but primrose grounds extend the colour-range to purplish-crimson. The one crimson-purple was difficult to place and may have had an ivory ground.

Summing the three Union Jack crosses, we have 348 individuals with anthocyanin to 92 without when expectation is 330 : 110. The evidence of the breeding experiments on anthocyanin flower colour clearly demonstrates the existence of two independent factors producing anthocyanin, one of which is tetrasomic.

(d) Mosaicism.

In mosaic forms the distribution of the anthocyanin is discontinuous in both stems and ray florets. It occurs in flecks and streaks and sometimes in larger areas (Plate IX, figs. 12 and 13). *The flavone ground of the rays is never mosaic.* The size of the coloured area may vary from one cell to the whole of the capitulum. Both the pale and deep pigments may be mosaic, but *the pale anthocyanin is never mosaic unless mosaic deep anthocyanin is present.* This is demonstrated particularly in families segregating forms with pale anthocyanin only, mosaic forms with deep anthocyanin only, and mosaic forms with both pale and deep. In this last class *both* pigments are indiscriminately mosaic; in the mosaic individuals with deep anthocyanin only, absence of the deep pigment reveals the clear yellow or ivory ground colour; but *all* the pale individuals are self-coloured, and crossed between themselves and with normal-coloured forms never give mosaics.

Two mosaic seedlings have been extensively used, namely M 5 and M 8. The colour of M 5 is crimson-scarlet and in the absence of this the ground is yellow, *i.e.* there is no trace of the pale anthocyanin. In 1926 M 5 was crossed with another mosaic, M 1 (purple on a magenta ground). From this cross (27/27) a number of mosaic and self-coloured individuals were raised. The self-coloured plants were of two kinds: (a) purplish or (b) apricots of various shades. Three of the purplish and four of the apricots were selfed and crossed with various seedlings known to be normal. 167 plants were raised from the apricots and *all of those with anthocyanin colouring were pale and normal.* The three self-coloured purplish seedlings were similarly crossed and 57 plants raised, all of which were normal.

It is clear therefore that (1) self-coloured derivatives of mosaic forms breed true for normal self-colour, and (2) pale-coloured forms do not give deep-coloured ones.

M 5 was further tested by crossing to a normal yellow seedling 14/26 (**Y₃yIi**). Twenty-two mosaic and 35 normal plants were raised (family

15/28), the normal plants comprising 33 yellow and 2 crimson individuals. Two ground colours and two corresponding anthocyanin colours were found in this family, one ground colour being a little paler than the other. On the paler yellow the anthocyanin appeared crimson; on the deeper yellow it was crimson-scarlet. No pale anthocyanin occurred. One of the self-coloured crimson seedlings (15^1/28) was back-crossed with its yellow female parent 14/26. The progeny (family 3/29) consisted of yellows and scarlets, and *all the forms with anthocyanin were moderately deep coloured and normal, no mosaicism occurring**.

This F_2 may therefore be taken to substantiate the true-breeding nature of self-coloured derivatives of mosaic forms. These two families 15/28 and 3/29 also disclose another fact—that M 5 is recessive for the **A** anthocyanin factor.

Including 15/28, six forms were raised in which M 5 was crossed to 14/26 (yellow) or White Star (ivory). No individuals with pale anthocyanin were found in a total of 263 plants. Moreover the ratio of individuals with anthocyanin to those without is 130 : 133. This must mean that M 5 is at least simplex for **B**, and its constitution for flower colour may be written provisionally as **YYyyI–aaaaBbbb**. Only two anthocyanin colours are found in these six families—crimson-scarlet and crimson. Empirical evidence strongly suggests that the factor **B** is cumulative in effect, and if this is so it is highly improbable that M 5 is duplex for **B**, otherwise deeper or paler colours than crimson and crimson-scarlet would be found in the progeny of M 5.

This tentative hypothesis has an important bearing on the nature of the inheritance of mosaicism in *Dahlia*, for M 5 gives a very close approximation to expectation on the assumption that it is simplex for **B**, therefore *the inheritance of mosaicism in Dahlia may be Mendelian* and entirely free from the effects of somatic segregation.

Before turning to the inheritance of mosaicism, another mosaic seedling M 8 (purple on pale magenta) must be mentioned. Crossed with the normal plants 14/26 (yellow) and White Star (ivory) it gave eight seedlings without anthocyanin in a total of 241. Four of these individuals were from crosses in which the female parent was without anthocyanin, and four from crosses in which the female parent had anthocyanin. Therefore all could not have arisen from accidental self-pollination. It is probable that these eight seedlings were an extreme condition of mosaicism, for among the progeny of M 8 twelve individuals were noted

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* In any appropriate family mosaic sectors are occasionally found, but these are clearly analogous with the type of sectorial mutation discussed later.

with only one or two solitary spots of anthocyanin, and in many others the coloured areas were very few. If this is so, then M 8 is triplex or quadriplex for **A**. Of the plants with anthocyanin 186 had the deep and 55 the pale pigment. Again it is possible that the pale individuals are extreme examples of mosaicism. Hence the significance of the ratio of 186 **BA** to 55 **bA** plants cannot at present be decided, but it is more probable that it indicates that M 8 is duplex for **B**, than simplex.

I have already stated that the deep pigment suffers considerable bleaching as the end of the growing season approaches. In two families, 28/27 and 33/27, both crosses between apparently normal plants, mosaics appeared. 28/27 gave 16 self-coloured, 6 mosaics, and 4 individuals which were scored as self-coloured early in the season, but later showed mosaic beneath the somewhat bleached deep pigmentation. Similarly in 33/27 the progeny consisted of 30 self-coloured, 1 mosaic, and 5 individuals showing mosaic at the end of the season. It is possible that these latent mosaics are due to the differential action of the mosaic factor upon the **A** and **B** pigments, but further work is necessary to establish this.

The inheritance of the mosaic character is shown in Table XI. The

TABLE XI.

Family	No. of plants	Parents		No antho-cyanin	Self-coloured deep an-thocyanin	Mosaic antho-cyanin	Variation of percentage antho-cyanin in mosaic seedlings	Average percentage antho-cyanin
46/30	53	14/26 (normal)	× M 5 (8–10 % anthoc.)	29	0	24	10–75	13
7/29	6	M 5 (12 % anthoc.)	× 14/26 (normal)	2	0	4	15–25	13
48/30	65	14/26 (normal)	× M 5 (50 % anthoc.)	31	3	31	1–70	15
47/30	65	14/26 (normal)	× M 5 (self-coloured) = 100 % anthoc.	28	6	31	10–70	22
45/30	17	White Star (normal)	× M 5 (self-coloured)	10	2	5	10–45	19
12/29	43	36/26 (normal)	× M 5 (self-coloured)	2	24	17	Trace–70	15
52/30	60	14/26 (normal)	× M 8 (12–15 % anthoc.)	0	8	46	Trace–90	31
51/30	41	14/26 (normal)	× M 8 (30 % anthoc.)	0	3	31	Trace–90	29
50/30	52	14/26 (normal)	× M 8 (self-coloured)	3	17	32	Trace–90	28
49/30	50	White Star (normal)	× M 8 (self-coloured)	1	4	29	2–75	20
9/29	12	M 8 (mosaic)	× Self	0	7	5	10–90	73

degree of mosaicism was determined by careful inspection of the whole of each plant at full bloom. The extent of the coloured area (referring to anthocyanin pigmentation only) is given as a percentage of the total area of the ray florets. In the case of M 5, capitula showing from approximately 10 to 100 per cent. anthocyanin pigmentation in the rays were used, and the total percentage of anthocyanin in the families ranged from 13–22. The total percentage seems to increase slightly with the percentage pigmentation in the parent capitulum.

The M 8 crosses were made with capitula showing 12–15, 30 and 100 per cent. pigmentation in the rays, but little variation was found in the results of each respective cross. The total percentage of pigmentation is considerably higher than for M 5, and this difference agrees with the difference in the average percentage of pigmentation characteristic for these two varieties, as estimated from observation. It is noteworthy that the family raised from selfing M 8 shows a high percentage of coloration, namely 73 per cent., as compared with the results when M 8 is out-crossed to a normal yellow-flowered individual.

The data from the breeding work on mosaic dahlias are not sufficient to warrant any certainty of opinion as to the inheritance of mosaic. Nevertheless the results show that the pale anthocyanin is only mosaic in the presence of mosaic deep pigment. Further, the segregation of forms with and without anthocyanin in the M 5 crosses seems to suggest that the inheritance of mosaic is factorial, the somatic variation seen in the rays apparently not extending to the germ cells. On the other hand, there are indications that the total percentage of anthocyanin in the families is related to the amount in the respective capitula used as parents.

COLOUR MUTATIONS.

Colour mutations in the petals of *Dahlia variabilis* are relatively common[1]. The mutation is usually confined to a narrow sector in a ray floret, the average width of the sectors being approximately one-eighth of an inch. These sectors may appear in the upper or lower epidermis[2], usually in one or the other, and only rarely do they coincide. The mutations may involve any of the pigments, their frequency being apparently the same for the yellow flavone and the two anthocyanins. The mutation of ivory can only be detected with reagents. Rarely as much as half or the whole of a floret may mutate, and in a total of 5000 plants the mutation of an area as large as a whole floret has been observed less than a dozen times. From the general distribution and nature of these mutations it appears probable that they are mericlinal chimaeras.

The fact that in the great majority of cases these sectorial changes are confined to the rays, and even then to a narrow sector reaching from base to tip, implies that the mutation occurs quite late and at a particular

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[1] The colour mutations referred to here are distinct from those constituting the phenomenon dealt with in a subsequent paper in this number of the *J. Genet.*

[2] There are three cell layers in a ray floret: (*a*) upper epidermis, (*b*) a middle layer of spongy mesophyll, and (*c*) the lower epidermis. The anthocyanins are apparently confined to the epidermal layers. The flavones occur throughout.

point in the development of the plant. It is probable that at this stage the internal conditions associated with cellular multiplication are such that mitotic irregularities attain their maximum. The evidence that the mutations are chromosomal (*i.e.* resulting from irregular distribution of part or all of certain chromosomes) is very strong, and on this assumption they have been of considerable value in confirming or indicating the factorial constitution of many plants[1].

On the supposition that the mutations are chromosomal it is apparent that, except when the factors are cumulative in effect, or two or more are involved simultaneously, loss mutation will be seen only in plants simplex for the mutant factor. Comparison of comparable families, however, shows that the frequency of mutation varies with the parents used. In some cases this is undoubtedly due to the large proportion of individuals which, in a given family, are simplex for several colour factors (cf. 29/29, etc.). The possibility of other causes modifying the mechanism of mitosis must not be overlooked.

The most conspicuous mutations are those involving the total loss of yellow or of the anthocyanins, and for this reason more of these kinds have been recorded, the less conspicuous changes being easily missed. It is significant that, wherever a change occurs in any shade of yellow (*e.g.* cream or primrose), it usually involves the *total* loss of that colour. Differences of intensity have been observed very rarely (Plate IX, figs. 7, 8). This is in agreement with the facts already established from breeding data, (1) that the **Y** factor is not cumulative in effect, the cream and primrose forms being due to the inhibition of the yellow pigment, and (2) that the inhibitors are usually in the simplex or duplex condition. Loss mutation of the inhibitors would therefore be difficult to detect. On the other hand, differences of intensity commonly occur in antho-

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[1] The loss or gain of one or more chromosomes in somatic divisions has been demonstrated in the following plants:

(1) *Hyacinthus orientalis* ($2n=16$). In Moreno, a triploid variety, Darlington (1926) found a somatic cell with $3n+1$ chromosomes. La Peyrouse, also a triploid, was found to have 25 and 26 chromosomes. In Totula, a tetraploid form, four divisions were found with 30 and three with 31 chromosomes, instead of the normal 32. De Mol (1921) had previously reported a similar loss in the same variety.

(2) *Crepis tectorum* ($2n=8$) (Navashin, 1930). Among the progeny of a triploid plant a $2n+1$ form was found which sported a $2n$ branch. The different branches were morphologically distinct. In this same species a case of chromosomal variegation was also reported, in which varying numbers of chromosomes were found in somatic tissue.

(3) *Narcissus odorus rugulosus* ($2n=14$). In a root tip preparation of this variety my colleague, Mr J. Philp, who is studying the cytology of this genus, has shown me a cell with 15 chromosomes. The adjacent cells have 14 chromosomes.

cyanin mutations, a fact supporting the idea that anthocyanin factors are cumulative in action.

Pale and medium magenta and apricot forms give only ivory and yellow sectors respectively, which is in agreement with seedlings simplex for **A**. When the pale and deep colours occur in the same flower, it not infrequently happens that the distribution of the pale colour is uniform, while the deep colour fades towards the tips of the florets, thus revealing the paler anthocyanin. Loss mutation of the deep colour in such flowers always discloses a paler coloration (Plate IX, fig. 9).

Occasionally all the anthocyanin pigment will be missing from a sector, while another immediately adjacent sector of approximately equal size will be found to be doubly pigmented. Thus an orange flower will show deep orange and yellow sectors side by side, the obvious inference being that in a plant simplex for **A** failure of equal distribution of the **A**-chromosome has resulted in sectors duplex and nulliplex for **A**. In individuals carrying more than one factor for anthocyanin similar conditions also result in adjacent intensified and diluted sectors.

If an ivory sector occurs in a plant simplex for **Y** and carries anthocyanin in addition, the flower colour will be magenta to purple instead of the usual apricot to scarlet, the precise intensity depending on the amount of anthocyanin present. It is noteworthy that in a number of cases the magenta or purple pigment seems much deeper than the apricot or scarlet.

In Table XII are presented some of the mutations recorded, along with the constitution of the plants or their parents. Examination of the table will show how far the occurrence of the mutations agrees with the hypothesis that they are due to the loss or gain of a chromosome or factor. Only the most important features of these mutations will be dealt with here.

The purple sectors in Union Jack are due to the loss of the **Y** factor: the tinged sector is probably due to the loss of the **B** factor. The yellow sector implies that both **A** and **B** factors have been lost. Other cases involving the loss of two or more different factors have been recorded in these experiments, and they would be expected occasionally if these mutations are due to irregular chromosome distribution at a particular mitosis. In Plate IX, fig. 10, an exceptional case is shown. The ivory sectors tested in Ideal confirm the deductions made from the breeding results that this variety is heterozygous for **I**.

The doubly deep sectors observed in $22^8/27$ are the result, presumably, of a division giving cells triplex or quadriplex for **A**. Family 6/28 bears

TABLE XII.

T.=tested.

Plants or Parents	Flower colour	Mutation
Union Jack ($Yy_3I_2Aa_3Bb_3$)	Crimson-scarlet	Purple sectors
Ditto	Ditto	Tinged sector
Ditto	Ditto	Yellow sector
Ideal ($Yy_3Iia_4b_4$)	Yellow	Ivory sectors. T.
$41^1/28$ ($Yy_3Iia_4b_4$)	Ditto	White or ivory sector
$22^8/27$ ($Y-y_2I_2A_2a_2b_4$)	Scarlet-orange	Doubly deep sectors
Union Jack ($Yy_2I_2Aa_3Bb_3$) selfed	Crimson-scarlet	Purple sector
$23/27 = 14/26 \times$ Union Jack	Scarlet	Purple sector
($Y_3yIia_4b_4$) $\times$ ($Yy_3I_2Aa_3Bb_3$)		
Ditto	Ditto	Yellow sector
2, $3/28 = 14/26 \times$ White Star	Primrose to cream	Ivory sector. T.
($Y_3yIia_4b_4$) $\times$ ($y_4Iia_4b_4$)		
Ditto	Primrose to cream (two)	White or ivory sector
Ditto	Primrose (two)	Ditto
Ditto	Yellow	Ditto
4, $5/28 = 14/26 \times 36/26$	Ditto	Ivory sector. T.
($Y_3yIia_4b_4$) $\times$ ($y_4I_2A?b_4$)		
Ditto	Tinged apricot	Magenta sector
Ditto	Apricot (two)	Ditto
$6/28 = 14/26 \times$ Ideal	Primrose	White sector. T.
($Y_3yIia_4b_4$) $\times$ ($Yy_3Iia_4b_4$)		
Ditto	Primrose (two)	Ivory sector. T.
Ditto	Yellow (three)	White sector. T.
Ditto	Yellow	Ivory sector. T.
$27/28 = 14/26 \times 22^8/27$	Orange	Yellow sector
($Y_3yIia_4b_4$) $\times$ ($Y-y_2I_2A_2a_2b_4$)		
Ditto	Scarlet-orange	Deeper sector
38, $39/28 =$ Union Jack $\times 14/26$	Crimson-scarlet	Yellow sectors
($Yy_3I_2Aa_3Bb_3$) $\times$ ($Y_3yIia_4b_4$)		
Ditto	Orange-scarlet	Very faintly tinged sectors
$40/28 = 35/26 \times 22^9/27$	Yellow	White sector. T.
($y_4i_2a_4b_4$) $\times$ ($Y_2y_3Iia_4b_4$)		
Ditto	Ditto	Ivory sector. T.
Ditto	Ditto	White or ivory sector
$41/28 = 32/26 \times 31^5/27$	Ditto	Ivory sector. T.
($y_4i_2a_4b_4$) $\times$ ($Yy_3Iia_4b_4$)		
Ditto	Ditto	White or ivory sector
$4/29 = 36/26 \times 32/26$	Deep rosy magenta	Doubly deep and dilute adjacent sectors. Also ivory sector. T.
($y_4I_2A?b_4$) $\times$ ($y_4i_2a_4b_4$)		
39, $40/29 =$ Union Jack $\times 34/26$	Scarlet-crimson	Yellow sector
($Yy_3I_2Aa_3Bb_3$) $\times$ ($Y_2y_2I_2a_4b_4$)		
Ditto	Orange-scarlet	Ditto
Ditto	Orange	Ditto
Ditto	Scarlet-crimson	Deeper and dilute adjacent sectors
$42/29 = 14/26 \times 22^8/27$	Crimson-scarlet	Doubly deep sector
($Y_3yIia_4b_4$) $\times$ ($Y-y_2I_2A_2a_2b_4$)		
Ditto	Orange	Yellow sector
Glenshee $\times$ Union Jack	Very faintly tinged apricot	Yellow sector
($Yy_3I_2Aa_3b_4$) $\times$ ($Yy_3I_2Aa_3Bb_3$)		
Ditto	Pale apricot	Pale magenta sector
Ditto	Orange (two)	Yellow sector
Ditto	Orange	Very faintly tinged sector
Ditto	Ditto	Apricot sector
Ditto	Ditto	Doubly deep and yellow adjacent sectors
Ditto	Ditto	Doubly deep sector
Ditto	Ditto	Magenta
Ditto	Ditto	Deeper and dilute sectors
Ditto	Scarlet-orange	Yellow sector
Ditto	Bleached scarlet (two)	Yellow and purplish sectors

TABLE XII (*continued*).

Plants or Parents	Flower colour	Mutation
Glenshee × Union Jack ($\mathbf{Yy_3I_2Aa_3b_4}$) × ($\mathbf{Yy_3I_2Aa_3Bb_3}$)	Orange-scarlet	Yellow sector
Ditto	Orange-scarlet (three)	Magenta sector
Ditto	Scarlet (three)	Purplish sector
Ditto	Ditto	Yellow sector
Ditto	Crimson-scarlet (two)	Ditto
Ditto	Crimson-scarlet	Faintly tinged magenta sector
Ditto	Crimson-scarlet (three)	Purple sector
Ditto	Crimson-scarlet	Adjacent very faintly tinged apricot and purple sectors
Ditto	Scarlet-crimson (two)	Yellow sector
Ditto	Scarlet-crimson	Purplish sector
Ditto	Ditto	Dilute sector
Ditto	Purplish-crimson	Ivory sector
Ditto	Crimson-purple	Ditto
Ditto	Purple (two)	Ditto
Ditto	Rosy purple	Ditto
Ditto	Rosy purple (three)	Pale magenta sector
Ditto	Magenta-purple	Doubly deep and dilute adjacent sectors
Ditto	Magenta	Doubly deep sector
9, 10/30 = $2^3/28 × 32/26$ ($\mathbf{Y_2y_2I_2a_4b_4}$) × ($\mathbf{y_4i_2a_4b_4}$)	Cream (two)	White sector. T.
20, 21/30 = $10^1/28 × 2^3/28$ ($\mathbf{y_4Iia_4b_4}$) × ($\mathbf{Y_2y_2I_2a_4b_4}$)	Primrose to cream	Ivory sector
Ditto	Ditto	Ivory sector. T.
Ditto	Cream	White sector. T.
Ditto	Primrose	Ditto
Ditto	Ditto	Ivory sector. T.
Ditto	Cream	Ivory sector
Ditto	Pale cream	Ivory sectors. T.
22/30 = $2^3/28 ×$ Everest ($\mathbf{Y_2y_2I_2a_4b_4}$) × ($\mathbf{y_4I_2a_4b_4}$)	Primrose (three)	Ivory sector. T.
Ditto	Yellow	Ditto
26, 27/30 = $10/29 × 2^3/28$ ($\mathbf{y_4I_2a_4b_4}$) × ($\mathbf{Y_2y_2I_2a_4b_4}$)	Primrose (two)	Ivory sector
Ditto	Yellow	Ditto
28/30 = $32/26 × 6^2/29$ ($\mathbf{y_4i_2a_4b_4}$) × ($\mathbf{Yy_3i_2a_4b_4}$)	Yellow	White sector. T.
29/30 = $32/26 × 14/26$ ($\mathbf{y_4i_2a_4b_4}$) × ($\mathbf{Y_3yIia_4b_4}$)	Primrose (two)	Ditto
Ditto	Ditto	Ivory sector. T.
Ditto	Yellow	White sector. T.
33/30 = $41^1/28 × 2^2/28$ ($\mathbf{Yy_3Iia_4b_4}$) × ($\mathbf{Yy_3Iia_4b_4}$)	Ditto	Ivory sectors. T.
36/30 = $41^1/28 × 41^2/28$ ($\mathbf{Yy_3Iia_4b_4}$) × ($\mathbf{Yy_3i_2a_4b_4}$)	Ditto	Ivory sector. T.
40, 41/30 = $2^3/28 × 41^1/28$ ($\mathbf{Y_2y_2I_2a_4b_4}$) × ($\mathbf{Yy_3Iia_4b_4}$)	Cream	Ditto
Ditto	Primrose to cream	Ditto
Ditto	Primrose (two)	White or ivory sector
Ditto	Yellow	Ivory floret. T.
Ditto	Ditto	White or ivory sectors
54/30 = $2^3/28 × 31^5/27$ ($\mathbf{Y_2y_2I_2a_4b_4}$) × ($\mathbf{Yy_3Iia_4b_4}$)	Primrose to ivory	White or ivory sector
61/30 = $32/26 ×$ Union Jack ($\mathbf{y_4i_2a_4b_4}$) × ($\mathbf{Yy_3I_2Aa_3Bb_3}$)	Deep magenta	Paler sector
Ditto	Ditto	Doubly deep and ivory adjacent sectors
Ditto	Bleached scarlet-crimson	Deep magenta sector
Ditto	Crimson	Yellow sector
58/30 = Glenshee × White Star ($\mathbf{Yy_3I_2Aa_3b_4}$) × ($\mathbf{y_4Iia_4b_4}$)	Yellow	Ivory sector. T.

out the postulated heterozygous constitution of 14/26 and Ideal for **I**.

The mutations in family 40/28 confirm the breeding results. The mutations in the family Glenshee × Union Jack indicate that in this cross different genotypes may be the same colour. Mutations of the **Y**, **A** or **B** factors are shown. The yellow sectors quite frequently found in scarlet-crimson, orange-scarlet, etc. florets are most probably due to the loss of the **B** factor in plants simplex for **B**.

The two white sectors recorded in families 9 and 10/28 and 20 and 21/28 indicate that some of the yellow individuals have white grounds, though in each family one of the parents is homozygous for **I**. The inhibition of ivory is therefore common in these families, but of course can only be measured satisfactorily when yellow pigment is absent.

Family 29/30 is important because the constitution of 14/26 for **I** has never been determined by breeding. The mutations clearly indicate that it is heterozygous, and taken in conjunction with the mutations in 6/28 there can be little doubt as to the correctness of this assumption. In addition to these mutant sectors several other capitula were tested, since the very considerable fading of the yellow pigment at the end of the growing season makes possible a direct test for the presence of ivory, which does not fade appreciably. These end-of-season tests gave approximately equal numbers of ivory and white grounds.

<div align="center">CHEMICAL.</div>

Tests made with the crude-flower pigments of *Dahlia* show that two distinct flavones and two distinct anthocyanins occur in this genus and in *D. variabilis.*

Willstätter (1915) found that certain deep brown-red varieties of *variabilis* form cyanin, but pelargonin is formed in the scarlet-red varieties. Both pigments were found in a dark violet form. Schmid and Waschkau (1928) isolated and analysed the yellow pigment of *variabilis* in quantity and stated it to be identical with apigenin. I have pointed out, however, that ivory flavone occurs in many, probably most, of the yellow varieties, and the question arises as to which of the pigments was isolated and identified, especially since apigenin is the ivory pigment in *Antirrhinum* (Wheldale and Bassett, 1913). As suggested by Wheldale, it seems probable that the yellow flavone is luteolin.

Hundreds of mosaic varieties and sectorial mutations have been recorded in these experiments, but no dahlia with anthocyanin colour has been found to have a white ground. This led me to the conclusion

that anthocyanin was not formed unless flavone was present, but it seems probable that my observations have not been sufficiently extensive to justify this opinion. Plants selected at random in these experiments proved to be of the following constitutions for **Y** and **I**. Yellows: Y_4 none, Y_3y two, Y_2y_2 five, Yy_3 eight. Ivories: **II** thirteen, **Ii** seven. Prof. J. B. S. Haldane, to whom I am greatly indebted, has worked out from these data the probability of the occurrence in a natural population of a coloured form nulliplex for both **Y** and **I**, and has provided the account which follows:

"Let u be the ratio of **Y** to **y** in the population, which therefore consists of $u^4Y_4 : 4u^3Y_3y : 6u^2Y_2y_2 : 4uYy_3 : 1y_4$. This is shown to be the distribution expected in a tetraploid population mating at random (Haldane, 1931). Similarly let it consist of $v^2II : 2vIi : 1ii$, this being the well-known equilibrium distribution in an outbreeding diploid. We have to find the best values of u and v to fit the observed ratios of genotypes among the dominants. Clearly $v = 26/7$ and u can be calculated from the method of maximum likelihood (Fisher and Balmukand, 1928). If the observed numbers be aY_4, bY_3y, cY_2y_2, dYy_3, we must choose u so that

$$a \log \left[\frac{u^4}{(u+1)^4 - 1} \right] + b \log \left[\frac{4u^3}{(u+1)^4 - 1} \right] + c \log \left[\frac{6u^2}{(u+1)^4 - 1} \right]$$
$$+ d \log \left[\frac{4u}{(u+1)^4 - 1} \right]$$

is a maximum. The condition for this is:

$$\frac{u^3 + 4u^2 + 6u + 4}{u(3u^2 + 8u + 6)} = \frac{a + b + c + d}{3a + 2b + c}.$$

This latter value is 15/9 in the present case. Hence $u = \cdot4474$, giving the following values:

	Y_4	Y_3y	Y_2y_2	Yy_3
Observed	0	2	5	8
Calculated	0·18	1·59	5·32	7·92

Hence the proportion of y_4 recessives is $\frac{1}{(u+1)^4}$ or $\cdot2278$, that of **ii** recessives $\frac{1}{(v+1)^2}$ or $\cdot0450$, while that of the **yyyyii** recessives is their product, or $\cdot01025$, *i.e.* 1·025 per cent."

We see then that individuals lacking **Y** and **I** but with anthocyanin present will constitute only 1 per cent. of a population, and in view of this it is extremely improbable that such forms would be discerned at all when their identification depends on fortuitous mutations

at rare intervals. This question of the independence of the production of anthocyanins and flavones has not been proved genetically in *Dahlia* because of the difficulty of isolating the material required for a conclusive test. Some of the requisite forms have now been isolated and it is hoped shortly to verify this hypothesis.

The reactions of flavones and anthocyanins with ammonia and sulphur dioxide were briefly stated in the earlier paper. I am indebted to Miss R. Scott-Moncrieff for the following method, by which additional tests are being made. Petals are first macerated in a dilute aqueous solution of hydrochloric acid. In this acid solution pure pelargonin gives an orange-red, pure cyanin a cherry-red colour. On the addition of a few c.c. of saturated aqueous solution of sodium carbonate the following colour reactions are found: pure pelargonin gives a red-violet, pure cyanin gives a clear green-blue. In *Dahlia* pelargonin with yellow flavone gives a red-brown, and pelargonin plus cyanin gives shades of red-brown-violet to violet-blue according to the proportion of pelargonin, cyanin and yellow flavone.

It was found that a magenta (ivory ground) gives the typical blue-green reactions for cyanin. A scarlet (yellow ground) gives the brown-red reaction due to the presence of pelargonin and the yellow flavone. Forms with cream or primrose grounds plus anthocyanin give reactions intermediate between blue-green and brown-red. Except possibly in pale varieties the proportion of ivory flavone to cyanin was insufficient to mask the cyanin colour reactions.

The yellow flavone is of so intense a colour both in the flower and in its reactions that it is practically impossible to detect the presence of ivory, and not at all easy to detect the presence of cyanin when yellow is also present. For example, it is not probable that the scarlet-flowered species produce the ivory flavone, yet when they are tested side by side with a variety of *variabilis* of precisely the same colour but known to be homozygous for **I**, the reactions are exactly alike. For this reason the colour of the flowers and the crude pigment reactions afford little indication of the relative *amounts* of the four pigments present.

Yellow, ivory and white varieties have green stems and foliage. Plants with anthocyanin in the rays invariably have anthocyanin in the stem. The intensity of the stem pigmentation approximates to that of the rays. The disc of pale or moderately anthocyanin-coloured varieties is usually yellow, but in forms with deeply pigmented rays anthocyanin is also found in the disc florets and bracteoles. However, a certain degree of independence occurs, and forms with (approximately) equally intense

rays may have discs with slight or pronounced pigmentation. Comparison of suitable material suggests that this pigmentation of the disc is associated with a general pigmentation of every part of the plant *as distinct from the ordinary ray floret coloration*. Should a different anthocyanin, or anthocyanins, be concerned in this general pigmentation, it would have to be taken into account in tests on the ray floret colours, which, being already intensely coloured, are scarcely affected by the lesser effects of the general pigmentation.

A detailed study of the reactions of the crude pigments is at present in progress. As far as the experiments have gone they indicate that:

(1) Cyanin is produced by the **A** factor in the presence of ivory flavone.

(2) When the **B** factor is also present with ivory, pelargonin, apparently in small quantities; is sometimes found.

(3) Orange and scarlet varieties give reactions which differ only in intensity.

(4) A general pigmentation of all parts of the plant is found in forms with deeply coloured rays, and it is probable that this additional pigmentation increases the difficulty of identifying the flower colour pigments.

The results of this part of the experiments will be dealt with on a later occasion in conjunction with further work on the inheritance of anthocyanin.

Incompatibility.

Self-incompatibility is general in *D. variabilis*, the occurrence of low degrees of partial fertility being exceptional.

Table XIII gives the gross results of self- and cross-pollinations made in these experiments. In the self-pollinations the total of 251 capitula is equal to at least 30,000 florets, giving a percentage of 0·04 seeds set to florets pollinated.

The fertility of $27^1/27$ and $27^3/27$ is quite exceptional. In view of the data from cross-pollinations made with $27^3/27$, it seems probable that this seed production can be ascribed to pseudo-fertility as postulated by East (1925) for *Nicotiana*. Nevertheless the possibility of sectorial mutations involving the loss or gain of incompatibility factors must not be overlooked. Leaf shape, colour patterns and the number of rays have all been observed to mutate in a similar way to the flower colours, and if such mutations are the visible result of chance chromosome irregu-

larities at a mitosis late in the growth of the plant, it is also possible that the chromosomes carrying the allelomorphs would be affected in turn.

As will be seen from Table XIII, all grades of fertility are found in cross-pollination. This indefinite variation cannot be altogether attributed to indifferent pollination, for care was taken to ensure in every respect as good conditions as possible at the time of pollination. Since *variabilis* is an allo-octoploid (*i.e.* double autotetraploid), it is probable that most factors will be duplicate and tetrasomic. The duplicate series would be

TABLE XIII.

Self-pollination of 54 different individuals.

157	capitula	gave	0	seeds
11	,,	,,	1	seed each
1	capitulum	,,	2	seeds
1	,,	,,	6	,,
1	,, $(27^3/27)$	,,	11	,,
1	,, $(27^1/27)$	,,	16	,,
Also 79	capitula	gave a total of	83	,,
=251	,,	,,	139	,,
=approx. 0·04 % seeds to florets				

Cross-pollination (240 different combinations).

87	capitula	gave	0	seeds
51	,,	,,	1–10	,,
32	,,	,,	11–20	,,
34	,,	,,	21–30	,,
31	,,	,,	31–40	,,
23	,,	,,	41–50	,,
29	,,	,,	51–60	,,
11	,,	,,	61–70	,,
18	,,	,,	71–80	,,
7	,,	,,	81–90	,,
2	,,	,,	91–100	,,
1	,,	,,	116	,,
Also 23	,,	gave a total of	304	,,
=349	,,	,,	8466	,,
=approx. 36 % gross cross-fertility				

mainly similar if not identical (cf. **A** and **B**, **Y** and **I**). Hence the incompatibility factors might be expected to constitute two independent, tetrasomic, multiple-allelomorphic series giving rise to great diversity in the behaviour of cross-incompatibility.

In these, as well as in self-pollinations, different capitula from the same plants have given different results in similar pollinations and the effects of pseudo-fertility are apparently localised. Since the external environment can have varied only in the slightest degree, it follows that this localisation of pseudo-fertility is mainly the result of local variation of the internal environment.

In several instances self-fertilisation has been induced in cross-pollinations. The individuals arising from such "crosses" can almost invariably be recognised at once by their dwarf stature, general weakness, late flowering, aberration of the disc florets (which become foliaceous) and other signs. In the examples to be quoted this is always the case unless otherwise stated.

The purple seedling M 8 (**yyyyIIA---B---**) pollinated by 22⁹/27 (**YYyyIiaaaabbbb**) gave 13 plants in F_1, all of which were purple. Expectation was, approximately, 11 seedlings with yellow grounds to 2 with ivory. This same purple was also pollinated by a deep purplish-crimson seedling, 39¹/28, with a yellowish ground. 38 plants were raised; only 28 could be scored for flower colour before the end of the season and of these 24 were purple, 2 magenta, 1 crimson and 1 apricot, the crimson and apricot having yellow grounds. These two plants and several of the purples were of normal stature and appearance, and were probably derived from cross-pollination. The remainder were undoubtedly from self-pollination. 39¹/28 is being tested further for the **Y** factor and is already known to be either simplex or duplex for **Y**.

27³/27 (apricot) has twice given progenies from self-fertilisation following cross-pollination. In the first case, 27³/27 (apricot) × 27⁹/27 (crimson-scarlet) gave 25 individuals, none of which was deep-coloured; 18 had yellow grounds, 6 ivory and 1 failed to flower. 27³/27 is the derivative of M 5 (**Yyyy**) × M 1 (**yyyy**) and is therefore simplex for **Y**. The ratio of 18**Y** : 6**y** strongly supports the origin by selfing of this family. In the second case 27³/27 was crossed by 14/26 (**YYYy**) and four plants very similar to 27³/27 were obtained.

In view of the evidence from selfing and crossing 27³/27 with co-incompatible varieties it seems highly probable that (1) pollen tube growth in this seedling is greater than in many other dahlias, and (2) the presence of foreign pollen either prolongs the life of the style or induces a reaction accelerating the growth of the 27³/27 pollen tubes, thus enabling a proportion of them to accomplish fertilisation. The same may be said of M 8 or any other dahlia exhibiting pseudo-fertility.

Two other instances of considerable interest may be mentioned:

(1) Everest (**yyyyII**) × White Star (**yyyyIi**) gave 19 plants of which 9 were approximately only half the height of the remainder. Everest has deep ivory single flowers with twisted petals, White Star has pale ivory semi-double flowers with flat petals. Singleness, flat petals and ivory colour are all dominant characters, and the first two are known to be polysomic and intermediate in the heterozygous types. The nine small

plants were all deep ivory with twisted petals, whereas the intensity of ivory varied in the remaining 10 plants which had more or less intermediate petals (Text-fig. 1). There is little doubt but that the 9 deep
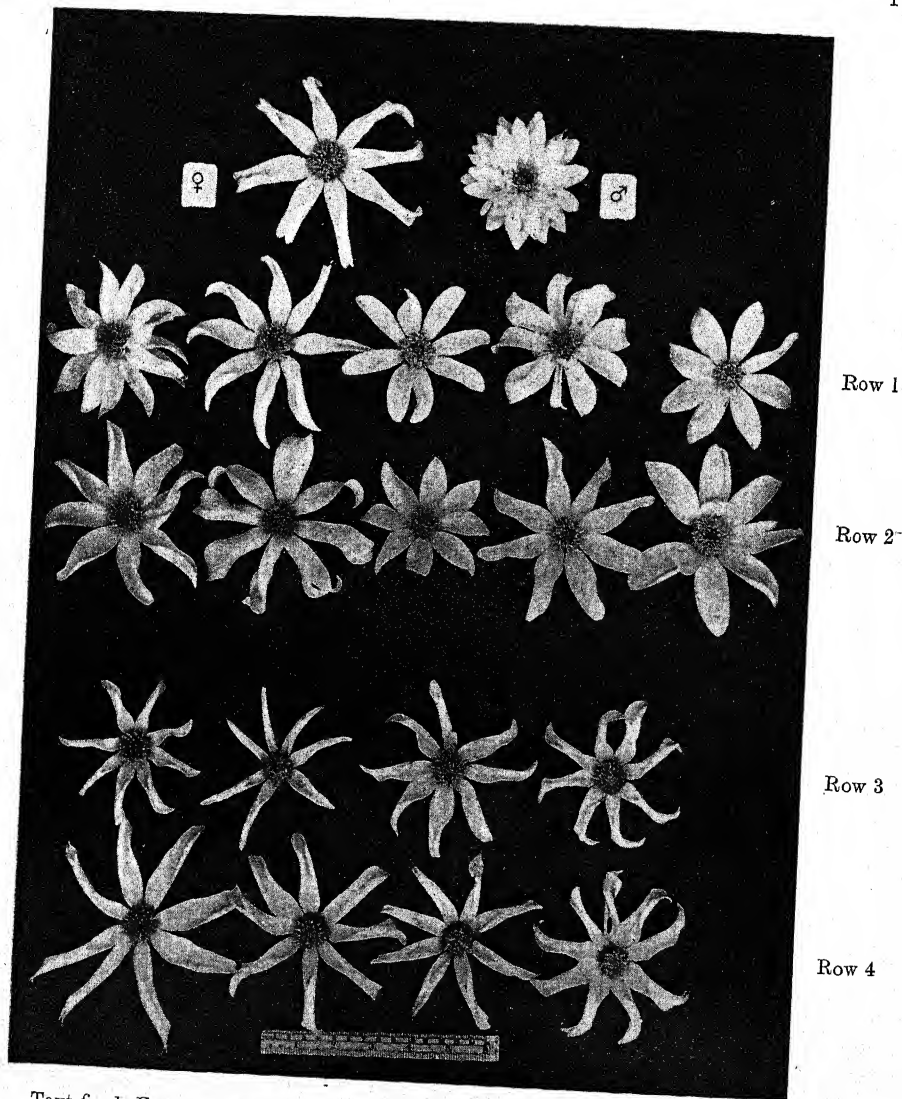
Text-fig. 1. Everest × White Star (rows 1–4). Rows 1 and 2: Capitula which arose from cross-pollination. The intensity of the ivory rays varies as does the degree of twisting which is less than in the female parent. Four capitula have more than eight petals. All from vigorous plants. Rows 3 and 4: Capitula which arose from self-pollination in the same cross. These capitula have deep ivory rays, much twisted petals, eight petals only and they came from stunted plants.

ivory seedlings resulted from selfing, were homozygous for **I**, and relatively homozygous for twisted petals.

(2) Nineteen capitula of White Star (**yyyyIi**), or approximately 2000 florets, have been selfed, but only two seeds obtained. In the course of the interspecific pollinations three capitula of White Star were pollinated by the scarlet-flowered *D. coronata*. These capitula gave 1, 3 and 11 seeds respectively. The F_1 was extremely weak, but 11 individuals subsequently flowered and *all had ivory rays*. These plants were unquestionably from selfing, and chromosome counts showed them to be approximately octoploid. They could not have arisen apogamously, since segregation occurred for leaf-shape and number and shape of petals. The **I** gametes evidently have some advantage over the **i** gametes.

Four plants of *D. coronata* and three of *D. coccinea* were tested for self-fertility and all were found to be self-incompatible. This is also shown by the complete failure of these species to set seed at the commencement of the flowering season when solitary or few flowers are in bloom. As the number of flowers increases seed production is more prolific, owing to the greater chances of pollination among the relatively few plants grown.

Seed germination in *D. coronata* is very good; in *D. coccinea* it is poorer. It is probable that a certain amount of gametic sterility occurs in this last species. *D. Merckii* is self-fertile.

CYTOLOGY.

The observations have been made on pollen mother-cells. Material was fixed in Carnoy-Flemming and Carnoy-2 B.E. (La Cour, 1931), sectioned in paraffin wax at 16μ and stained by the gentian-violet method. Drawings were made at bench level with the aid of an Abbé camera lucida, a Leitz 2 mm. objective (n.a. 1·4) and a Zeiss × 30 compensating eyepiece to give a magnification of 3700. All drawings were reduced to $\frac{3}{4}$.

I am indebted to Mr L. La Cour for the preparation of the material.

The somatic chromosome numbers of *Dahlia* species I have examined are as follows:

D. coronata	32
D. coccinea	32
D. Maxoni	32
D. imperialis	32
D. Merckii	36
D. variabilis	64 (octoploid)

The evidence for the conclusion that 32 and 64 are tetraploid and octoploid numbers respectively was stated in the preliminary paper, and is derived from the types of association found in *D. variabilis*.

A detailed account of the chromosome behaviour of these and other composites is in preparation under the title "The Secondary Association of Chromosomes," but in the present account only those facts relevant to the discussion will be surveyed.

The reduction divisions in *D. variabilis* are remarkably regular, a possible laggard having been seen only once. This great regularity was not expected in so high a polyploid, but it is now clear that it is due to a special feature of the behaviour of the chromosomes, *i.e.* the complete terminalisation of all chiasmata. Only two types of configurations are found. These are figured as seen, and diagrammatically, in Text-fig. 2. The first type (*a*) has a single terminal or nearly terminal chiasma, and terminal or nearly terminal attachment to the spindle fibre. The second
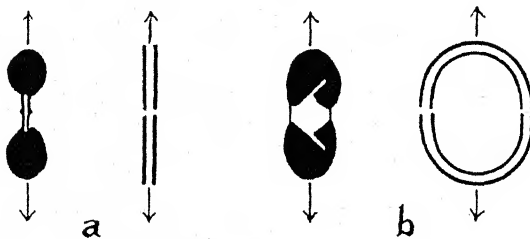
Text-fig. 2. The two types of bivalent configuration found in *Dahlia*. (*a*) terminal chiasma and attachments; (*b*) terminal chiasmata and median attachments.

type (*b*) has two terminal chiasmata and median attachments. The occurrence of only these two types of configuration precludes any possibility of irregularity in disjunction. This is true even in multivalent associations which are of the ring type with the components orientated symmetrically across the equator.

Neither chain and cross configurations nor interstitial chiasmata have ever been seen. The results of this extreme meiotic regularity are reflected in the very high percentage of good pollen and in the seed germination—the latter frequently attaining 100 per cent.

The (*b*) type bivalents with two terminal chiasmata are always the last to disjoin at anaphase. As Newton and Darlington (1929) have suggested, this is probably due to the greater length of the chromatids which separate at anaphase, as compared with the minimum of chromatid separation in the (*a*) type with a single terminal chiasma.

A conspicuous feature of meiosis in *D. variabilis* is the frequent association of bivalents in groups of two or more at the metaphase of the first division (Text-fig. 3). This association varies from the most intimate to a point where it ceases to be apparent, and for various reasons it cannot be ascribed to bad fixation. First, it is not found in diploids; secondly, the number and size of the associations is limited at a characteristic value for a particular species. Moreover the property of selection is an inherent quality of this type of association, chromosomes of like configuration being found in juxtaposition.

A certain percentage of these associations can be shown to be the result of pairing at zygotene of four or more chromosomes, giving rise to the typical quadrivalent or multivalent associations found at metaphase of the first division in polyploids.

Many of the associations seen in *D. variabilis*, however, are not the result of prophase pairing, but originate at pro-metaphase. We may
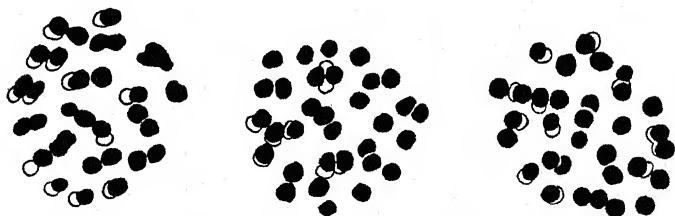
Text-fig. 3. Primary (multivalent) and secondary chromosome association in *D. variabilis* ($2n = 64$). Polar views—first metaphase.

distinguish such associations from *primary* or prophase pairing by the term *secondary* (or post-synaptic) association.

Cytological observations by McClung on *Mermiria* (1917), Seiler on *Phragmatobia* (1926), Belling on *Datura* (1928), and Darlington on *Tradescantia* (1929), have shown that pairing of the chromosomes at metaphase is between *parts* of chromosomes. The prophase basis of these observations is given by Newton and Darlington (1929), who have shown that pairing of chromosomes at prophase is not between chromosomes as wholes but between the particles of them which are homologous and presumably identical. Darlington (1930) has further shown that the maintenance of prophase pairing is due solely to the formation of chiasmata, *i.e.* the interchange of *chromatid* partners.

It follows therefore that chromosomes which are paired (*i.e.* materially associated) at metaphase of the first division must have been similar in a sufficient proportion of their length to permit of the formation of

chiasmata. But chromosomes which are identical only in minor and scattered portions of their length, so that they will be unable to maintain or even form chiasmata, may yet have a general affinity which would result in their attraction to one another at certain stages of meiosis. We see then that there are two types of association possible at meiosis, and in particular at metaphase of the first division: (*a*) pairing of homologous chromosomes which arises from a prophase relationship and persists to first anaphase, and (*b*) associations which arise from the general affinity of relatively homologous chromosomes, resulting in their juxtaposition at first metaphase. In the first case there will be material connections between the homologues which segregate according to the structural identity and relation of the components of the association. This is *primary* association. In the second case there will be no material connections, *and segregation will be unaffected by this secondary association.*

Now at diakinesis in plants the individual chromosomes and chromosome associations are repelled to the maximum degree, and only those connections which arise from prophase pairing persist through this stage to first metaphase. Hence secondarily associated chromosomes are never found in diakinesis—the requisite conditions for their association not occurring until pro-metaphase, when the chromosomes are in very close proximity preceding the "line up" for division. Both types of association may occur in the same plant—the degree of secondary association being particularly variable.

We see then that the secondary association of chromosomes is an invaluable criterion of the general relation of the relatively homologous sets found in *allo*-polyploids. The fact that two plants will cross to give even a sterile hybrid implies a certain relation between them, and since the chromosomes carry the fundamental units of heredity which determine the identity of an individual, they too must be structurally (morphologically) related. That they are recognisably so has been demonstrated in numerous allied species. All fertile allo-polyploids are the result of the crossing of two related but differentiated species, hence we should expect *a priori* to find secondary pairing in such hybrid polyploids.

In *D. variabilis* quadrivalents and occasional sexivalents are seen at diakinesis. The average number of quadrivalents at this stage is not easy to determine, but it is at least four per cell. I have never seen more than one sexivalent per cell at diakinesis (but cf. Table XIV (*b*)).

Table XIV gives (*a*) the total number of associations, primary and secondary, found in twelve metaphase plates, and (*b*) the number of probable multivalent associations in the same cells. The total number

of associations is certainly under-estimated, since only the obvious groups were relegated to their respective classes, all others being reckoned as a lower order of association than was probably the case. The primary associations are probably slightly over-estimated owing to the difficulty of deciding the nature of the groups as seen in polar view. The difference in the number of associations found at diakinesis and first metaphase definitely points to a considerable amount of secondary association in *D. variabilis*.

The mean number of quadrivalents seen at diakinesis or metaphase is not a direct measure of the number of independent factors or linkage groups which will be found to exhibit tetrasomic segregation. A given factor will show tetrasomic segregation so long as four chromosomes

TABLE XIV.

(a) Primary and secondary association

Number of bivalents

(b) Primary association
Types of configurations

4 pairs	3 pairs	2 pairs	1 pair	Octa-valent	Sexi-valent	Quadri-valent	Bivalent
—	2	9	8	—	1	6	17
—	2	11	4	—	2	9	8
1	3	5	9	—	1	7	15
—	2	12	2	—	1	5	19
—	2	10	6	—	—	5	22
1	2	7	8	—	1	6	17
—	1	12	5	—	1	7	15
—	3	8	7	—	2	6	14
—	3	9	5	—	—	10	12
1	2	9	4	—	1	7	15
—	3	8	7	—	2	5	16
—	3	7	9	—	1	7	15

Mean no. of bivalents of each type of association:
| 0·25 | 6·99 | 17·95 | 6·16 | 0·0 | 3·24 | 13·33 | 15·41 |

regularly pair at random at the locus of that factor, and the pairing is followed by regular disjunction of the *bivalent* components of the quadrivalent set. A relatively slight differentiation of the two bivalent components of a quadrivalent set will considerably lessen the chances of chiasma formation between all four chromosomes, thus leading to bivalent in place of quadrivalent formation.

The most valuable criterion of homology is the maximum association. The highest degree found in *D. variabilis* was figured in the previous paper (Text-fig. 2 *h*, p. 147). Association of the chromosomes in *Dahlia* persists into anaphase of the second division; and in the left-hand second-division metaphase figured, three groups of three chromosomes each, ten groups of two chromosomes and three separate chromosomes are clearly seen. The components of one of these groups of two chromo-

somes are loosely associated. The equal number of groups of three and solitary chromosomes is significant and will be referred to again. Only once have more than three "sexivalent" associations been found in one division, but the occurrence of three is common.

THE HYBRID *D. VARIABILIS* (8 *n*) × *CORONATA* (4 *n*).

Numerous attempts have been made to cross the *Dahlia* species grown at Merton. All four combinations have been made between *DD. variabilis, Merckii, coccinea* and *coronata*, and all failed with the exception of *D. variabilis* × *D. coronata* (the scented dahlia).

In 1928 two capitula of 36/26, a magenta variety of *D. variabilis*, were pollinated by *D. coronata*. One seed was obtained which gave rise to a vigorous plant with flowers almost identical in colour with those of the male parent (Plate IX, figs. 14–16). In most other respects this hybrid (2/29) is intermediate in character (Text-fig. 4). It attains a height of 8 feet, however, where both parents are shorter. It has the characteristic sweet scent of *coronata* and is almost totally sterile. The absence of fruiting capitula considerably extends the flowering season of the hybrid. One of the first capitula produced showed a mutant sector on the underside of a ray floret—the colour being a deep magenta in place of scarlet-orange. Cytological examination showed this hybrid to be, as expected, a hexaploid ($2n = 48$). The colour-range of *D. coronata* is from scarlet-orange to scarlet. Differences in flower size, colour, leaves, etc. are found in minor degree. This species breeds true for the presence of yellow ground and anthocyanin. If *D. coronata* is tetrasomic for anthocyanin and yellow-flavone factors then the hybrid would be **BBb'b'YYy'y'I'I'**, the indices denoting the factors contributed by *D. variabilis*. On this provisional scheme the two **B**-chromosomes must have been lost in a mitotic division, thus making visible the effect of the ivory ground.

The same interspecific cross was repeated in 1929 and again one hybrid, 2/30, was raised. This closely resembled the first hybrid but was not so tall and had broader rays—differences probably due to a different combination of factors received from the female parent.

From the many scores of capitula which developed on the first hybrid in 1929, 16 seeds were obtained from open pollination. Ten of these germinated, giving seedlings of varying grades of vigour. Six of these flowered: four had scarlet-crimson rays, one was orange and the other purple. A preliminary count of the somatic chromosomes had shown these seedlings to have from 56 to 64 chromosomes, and it is practically certain that they are octoploids from natural back-crosses with *D. variabilis*.

Four of these six plants proved perfectly fertile; the others flowered too late for their fertility to be established with certainty.

A hexaploid of the constitution postulated for 2/29 would produce gametes with from 16 to 32 chromosomes, and it is highly probable that the six natural seedlings arose from union of gametes of the hexaploid
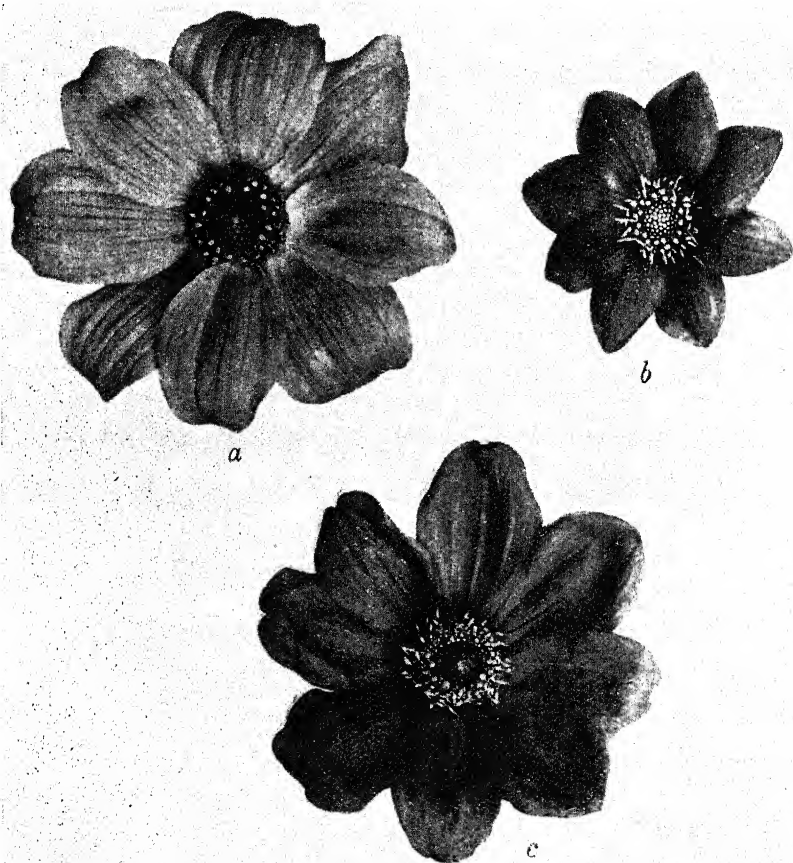
Text fig. 4. Capitula from—(a) *D. variabilis*, seedling 36/26; (b) *D. coronata*; (c) 2/29, = *D. variabilis* 36/26 × *D. coronata* (see also Plate IX, figs. 14–16).

containing the full *variabilis* complement with the normal tetraploid gametes produced by the octoploid species.

Pollen mother-cells of *D. coronata* regularly show 16 bivalents. No quadrivalents were seen in over 100 cells examined at diakinesis (Text-

fig. 5 *a*). Disjunction is very regular. The configurations are of two kinds as in *D. variabilis*, but secondary association at metaphase is much less pronounced (Text-fig. 5 *b–e*). It occurs nevertheless, and, if the orientation and disposition of the bivalents are taken into account, the metaphase plates will often reveal six to eight groups of two bivalents each. The looseness of the associations often renders them inconspicuous.

In *D. variabilis* the bivalent members of each association seen in side view are almost invariably at the same level. In *D. coronata*, however, some secondary associations are not quite symmetrical, the bivalent members being on different levels. It is possible that these asymmetrical associations are due to differences in the structural identity of the
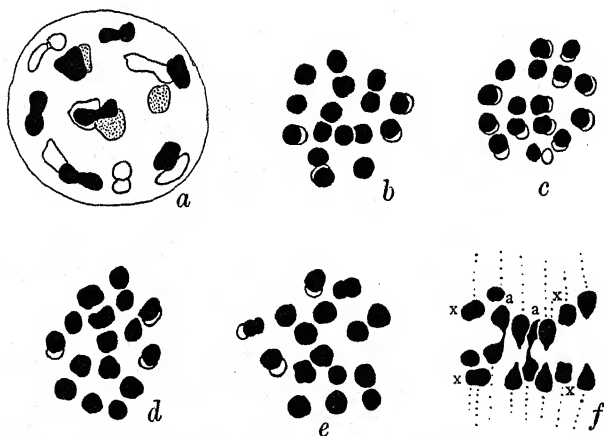
Text-fig. 5. *D. coronata* (2*n* = 32): (*a*) diakinesis, no association of bivalents; (*b–e*) polar views, first metaphase, showing secondary association of bivalents; (*f*) side view, early 1st anaphase (incomplete) showing the two types of chromosome configuration found in *Dahlia*. The pairs marked with a *x* have two terminal chiasmata; the others a single terminal chiasma.

components, homologous parts being somewhat differently located in each of the two chromosome pairs. This view is supported by the failure of the homologous bivalents to form quadrivalent associations, a failure which may be attributed to structural variation between pairs of chromosomes in a hybrid tetraploid. The absence of quadrivalents and the low degree of secondary association found in *D. coronata* suggest that this species is strongly allo-tetraploid.

The reduction divisions of the hybrid 2/29 are very irregular, univalents often lagging on the equator (Text-fig. 6). Association of the chromosomes occurs in high degrees, in consequence of which most of the observations have been made on polar views or single chromosomes in

side view. Careful examination of the associations, taking into account
the apparent number of bivalent and univalent components, shows that
the formation of trivalents is very common. Quadrivalent and sexivalent
associations are frequently seen in varying numbers (Text-fig. 7). The
number of univalents varies from 3 to 14 and these are nearly always
on the periphery of the spindle where they are easily recognised by their
greater transparency. The average number of univalents is about 6–9
per cell. The fact that there are never more than 14 univalents shows
that a proportion of the *coronata* chromosomes always pair with the
homologous types contributed by *variabilis*. In side view the chromo-
somes are seen to be very irregularly distributed, the univalents in
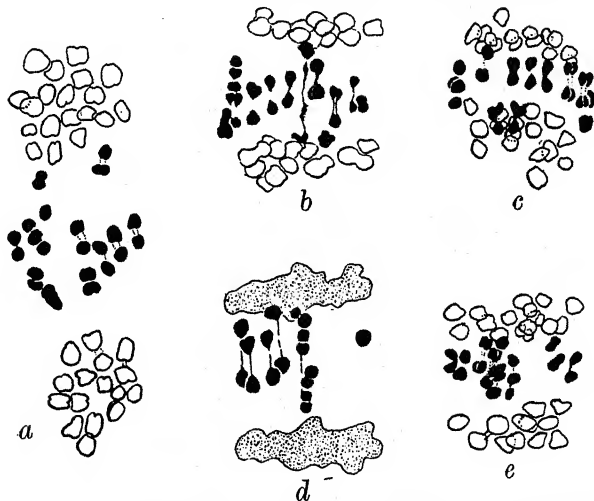
Text-fig. 6. Side views, first anaphase showing laggard univalents in the hexaploid hybrid
(2*n* = 48) *D. variabilis* × *D. coronata*: (*a–c*) 12 univalents, some dividing; (*d*) 7 univalents,
six dividing, two of which are constricted; (*e*) about 10 univalents.

particular often lying far off the equator (Text-fig. 7 *h*). These may divide
at the first anaphase or pass undivided to the poles.

The formation of a variable number of pairs between the chromosomes
of the two parental sets has been reported to occur in interspecific
hybrids by a number of workers. Avery (1930) gives a list of hybrids
in which this variability has been noted, but adds that "No interpretation
in accordance with modern genetic and cytological knowledge has yet
been suggested which seems adequate to account for the occurrence of
the variable pairing...."

Variable pairing in hybrids, however, is adequately explained by the
chiasma theory of pairing (Darlington 1929, 1930, 1931). On this theory

chiasmata arise at random between homologous and presumably identical chromosomes, the mean chiasmata frequency varying according to chromosome length. But where structural differentiation is considerable, so that only a small part or parts of the pairing chromosomes can pair to form a chiasma, then the mean number of chiasmata may be so reduced that in a proportion of cases pairing will fail.
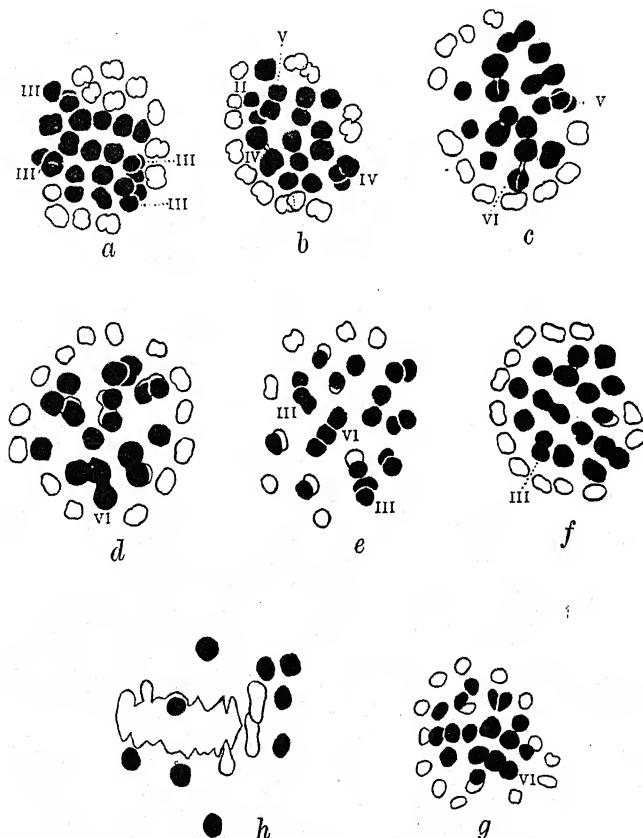
Text-fig. 7. (*a–g*) Polar views of first metaphase in the hexaploid hybrid (2*n*=48) *D. variabilis* × *coronata* showing univalents distributed on the periphery of the spindle and multivalent association among the bivalents; (*h*) side view, first metaphase showing irregular distribution of 9 univalents.

Chiasma frequency therefore is a function of the length of the chromosomes paired, and the degree of differentiation of the relative homologues in interspecific hybrids inevitably modifies this frequency—hence the number of bivalents or multivalents formed.

The most conspicuous feature of the cytology of the hybrid is the *large majority of bivalent and multivalent configurations of types which are never seen in either parent.* Text-fig. 8 shows some of these configurations. Text-fig. 8 *a* illustrates a bivalent with an interstitial chiasma. As I have shown, in *D. variabilis* terminalisation is complete or practically so. The occurrence of a bivalent with an interstitial chiasma in the hybrid is therefore due to failure of terminalisation, probably brought about by
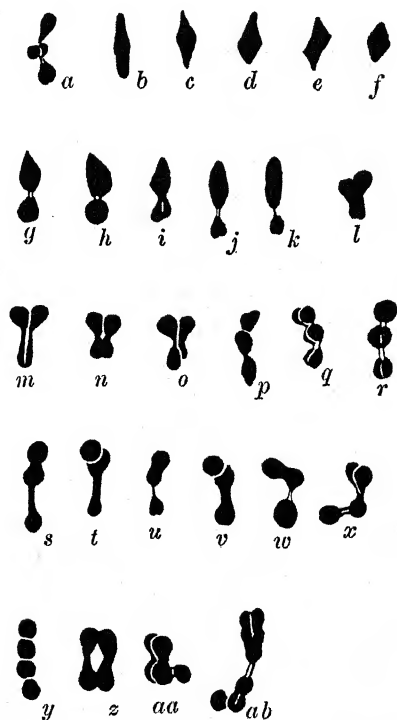
Text-fig. 8. Anomalous chromosome configurations from first metaphase of the hybrid *D. variabilis × coronata*: (*a–h*) bivalents; (*i–x*) trivalents; (*y–z*) quadrivalents; (*aa*) quinquevalent; (*ab*) sexivalent.

linear differentiation in the pairing chromosomes. In other words, the formation of an interstitial chiasma is evidence of hybridity. Text-fig. 8 *b–f* are other examples of bivalents with interstitial chiasmata. This type is extremely common in 2/29. Figs. *g* and *h* show unequal bivalents, such as are never seen in the parents. Figs. *i–x* represent trivalents of various configurations. Figs. *j–o* are complex forms, but most of the other trivalents figured are terminally associated. Fig. *y* shows a chain

of four chromosomes: Fig. *z* a quadrivalent as found in *variabilis*. Fig. *aa* is a quinquevalent type which occurs quite frequently. The last figure is of a sexivalent association. With the exception of types *a, y, z* and *ab* all these associations are commonly seen in side view.

The appearance in the hexaploid hybrid of these anomalous chromosome configurations may be assumed to show that homologous chromosomes contributed by *variabilis* and *coronata* pair in the hybrid, the unusual configurations being due to structural dissimilarities of the homologues. One such difference is shown in Text-fig. 5 *f*. In this side view two pairs of chromosomes marked *a* have their points of attachment close to the chiasma, giving the characteristic configuration shown. This type has not been found in *variabilis*. The preponderance of the anomalous configurations implies a general differentiation of the chromosome complements of the two parent species, which, however, has not proceeded far enough to prevent pairing. It is probable that in the absence of identical pairs autosyndesis occurs within the *variabilis* chromosome complement in the hexaploid, and that some of the configurations are due to this autosyndesis. That such is not always the case is shown by the varying number of univalents found, some of which must often successfully compete in pairing with the *variabilis* bivalents. Thus the large number of univalents seen in some plates is due to the reduction of zygotene pairing as a result of competition between homologous chromosomes.

It has not been possible to make an accurate estimate of the number of multivalent associations, but they are more frequent than in *D. variabilis*. Tetrad formation is surprisingly regular.

The cytology of the hexaploid hybrid *variabilis* × *coronata* indicates (*a*) that the chromosomes of these species are relatively homologous, often pairing at prophase, but (*b*) they are also structurally differentiated, as a result of which many anomalous configurations are found in the multivalent associations of the hybrid.

These species, though differing in chromosome number, may therefore be said (in the strictest sense) to be closely related.

DISCUSSION.

Scheme of phylogenetic relationship.

The origin of polyploid species under natural conditions is a phenomenon of paramount importance insomuch as a great number, perhaps

the majority, of plants are polyploids. Moreover it becomes increasingly apparent that the rôle of polyploidy in the evolution of new, vigorous species with a potential range of variability is a factor of considerable importance in the study of many cultivated plants and fruits. Very little genetic work on heterozygous allo-polyploids has been done. The studies on *Dahlia* are believed to present experimental evidence of a conclusive nature regarding the constitution and origin of a natural hybrid-polyploid. The first evidence for the hybrid origin of *D. variabilis* is as follows:

(1) With the exception of *D. variabilis*, *Dahlia* species may be arranged in two flower-colour groups: (a) ivory or magenta, (b) yellow, orange or scarlet. Both series of colours occur in *D. variabilis*.

(2) Four species cytologically examined were found to be tetraploids; a fifth was hypertetraploid. *D. variabilis* is an octoploid.

(3) The breeding evidence suggests that *variabilis* is a double auto-tetraploid. This is the most probable constitution for a hybrid of two tetraploid species after duplication of the hybrid chromosome complement has occurred.

Two factors, **Y** and **A**, are shown to be tetrasomic. A third, **I**, is almost certainly carried by four chromosomes, which pairing auto-syndetically, I_1 with i_1 and i_2 with i_2, give three zygotic types, $I_1I_1i_2i_2$, $I_1i_1i_2i_2$ and $i_1i_1i_2i_2$—the numerals indicating the pairing chromosomes. The fourth factor **B** has not been studied in the duplex condition which gives the critical tetrasomic ratios, but the general evidence also points to this factor being tetrasomic.

In regard to flower colour it is significant that of the five tetrapoid species I have grown, the three belonging to the ivory-magenta group have pale anthocyanin. The two species in the yellow-orange-scarlet group both have scarlet-orange flowers, the anthocyanin being of a greater intensity than that of the other three species.

D. dissecta is described by Watson as "purple or 'mauve-coloured'": *D. pubescens* as having purple flowers. The other two species in Group I have relatively pale-coloured flowers. Two other species in the yellow-orange-scarlet group have scarlet-orange and yellow rays (absence of anthocyanin) respectively. Apparently therefore the anthocyanin coloration in the ivory-magenta group is usually pale, but that of the yellow-orange-scarlet group is considerably deeper. This observation is of considerable import when we remember that the **A** factor produces pale and the **B** factor relatively deep pigmentation.

On the assumption that *D. variabilis* has arisen from hybridisation

of two tetraploid species, one belonging to each group, the scheme for flower colour may be represented as follows:

Group I		Group II	
Tetraploid species with magenta rays		Tetraploid species with scarlet rays	
AI		**BY**	

4n hybrid (presumably sterile)
ABIY

Fertile 8n hybrid

AI	**AY**	**ABY**	**ABI**	**BI**	**BY**
*magenta	apricot	deep scarlet	deep purple	purple	scarlet
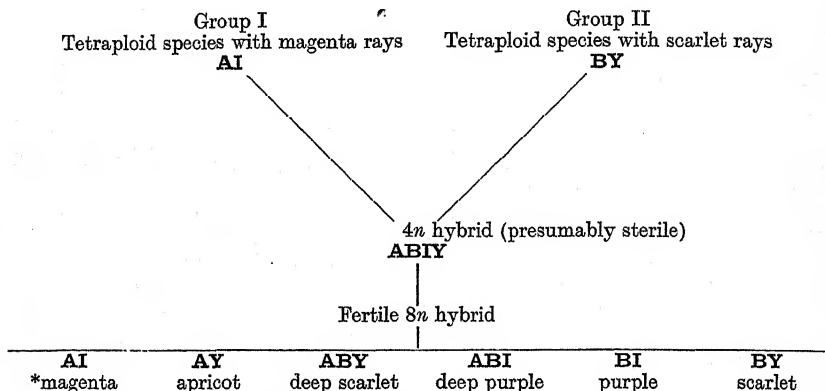
* These colours in the 8n hybrid are typical of those given by the combination of the factors **A**, **B**, **Y** and **I** in the simplex condition.

We see therefore that apricot, deep scarlet, deep purple and purple will be new colours from *recombination* of the colour factors.

The action of inhibitors in the tetraploid progenitors of *variabilis* would be simply to dilute the flower colour. But in the presence of both **Y** and **I** the action of these inhibitors on **Y** extends still further the colour range, giving various shades of crimson in the deeper colours and a curious colour intermediate between magenta and apricot in the lighter colours.

This is because flower colour in the dahlia results from the mixture of two series of soluble pigments. Anthocyanin with ivory flavone appears magenta or purple; with yellow flavone it appears scarlet. If production of the yellow flavone is partially suppressed, thus giving a cream ground, then when anthocyanin is present the flower colour will be intermediate, *i.e.* a crimson shade if much anthocyanin is present or magenta-apricot if there is only a little anthocyanin.

In the preliminary paper the first flower-colour group was given as ivory-magenta-purple. The experiments suggest, however, that the colour scored as purple is different from the mauve and purple colours of the species. The colour scored as purple in the experiments on *D. variabilis* is of considerable depth and intensity. The deepest of the **A** magentas (Plate IX, fig. 4) and a typical **B** purple (Plate IX, fig. 9) are shown in the colour plate. The deepest magenta never even approximates to the "palest" purple, the two colours being radically different.

It seems probable therefore that the purple of *D. dissecta* (as indicated by Watson's "mauve-coloured") may not be comparable with the deep pigmentation I called purple in *D. variabilis*. I have never seen living material of *D. dissecta* or *D. pubescens*, but dried specimens in the Kew Herbarium, though considerably faded, suggest that the flower colour is by no means as deep as my "purple." The flower-colour range in group I is therefore given as ivory to (deep) magenta.

We may now consider the general phylogenetic scheme as postulated on the results of the breeding work, morphology and cytology.

Most, if not all, fertile tetraploid species[1] of Dahlias must be allo-tetraploids, and therefore descended from diploid ancestral forms. In the descent of the tetraploid progenitors of *D. variabilis* differentiation occurred and among other distinctions gave rise to the two flower-colour groups. As the basis of visible somatic variation is in the structural differentiation of the chromosomes, the cumulative effect of those differences would bring about differential pairing of the homologues. Hence the tetraploid hybrid would be relatively sterile through failure of pairing between the homologues from the parents, and only doubling of the chromosome complement would restore fertility.

In Text-fig. 9 the chromosome complements derived from each of the tetraploid progenitors of *variabilis* are represented by the letters *A–H*. Associations of four bivalents are occasionally found at first metaphase, but never more than one. This chromosome type is represented by the letter *A*. The eight *A* chromosomes are the least differentiated of any of the eight types. Three associations of six bivalents are commonly found (types *B*, *C* and *D*). Association of six chromosomes leaves one bivalent unassociated, and it is possible that the three sexivalents and three bivalents seen in the second metaphase referred to on p. 289 are related in this way. Types *E*, *F* and *G* form primary or secondary quadrivalent associations. The *H* type is most differentiated. *H* and *H'* form bivalents only. *H''* forms quadrivalents.

The arrangement of the chromosome types in the figure is somewhat arbitrary and does not indicate their derivation. It is possible that *D. coronata* or a closely related species was the group II parent of *variabilis* as shown by the close structural relation of the chromosomes

[1] Complete terminalisation plus terminal and median spindle-fibre attachment as found in *Dahlia* chromosomes constitute a mechanism promoting regularity of the meiotic processes in polyploids. Such a mechanism might very appreciably increase the fertility of an auto-tetraploid. The cytological evidence (Lawrence, 1931) from *D. coronata*, *D. coccinea* and *D. Merckii*, however, does not support the view that these tetraploid *Dahlias* are auto-tetraploids.

of these species in the hybrid *variabilis* × *coronata*. Should doubling of the chromosome complement of this hybrid occur, we should have a fertile dodecaploid hybrid very closely related to *coronata* and *variabilis* but sterile with both species.

It is clear from the breeding results that the anthocyanin and flavone factors are carried by chromosome types which pair in the manner shown in Text-fig. 7. The occurrence of yellow and scarlet, ivory and magenta flower colours in opposite groups suggests that yellow replaces ivory, and deep replaces pale pigmentation, in group II. In other words,

Diploid ancestral stock
$n=8$

Hybrid diploid species

$n=8$

Group I tetraploids   Group II tetraploids
$n=16$ $n=16$

Tetraploid hybrid (presumably sterile)
$n=16$

Fertile octoploid species
D. variabilis
$n=32$

AAAA AAAA
BBBB BBB'B'
CCCC CCC'C'
DDDD DDD'D'
EEEE E'E'E'E'
FFFF F'F'F'F'
GGGG G'G'G'G'
HHH'H' H''H''H''H''
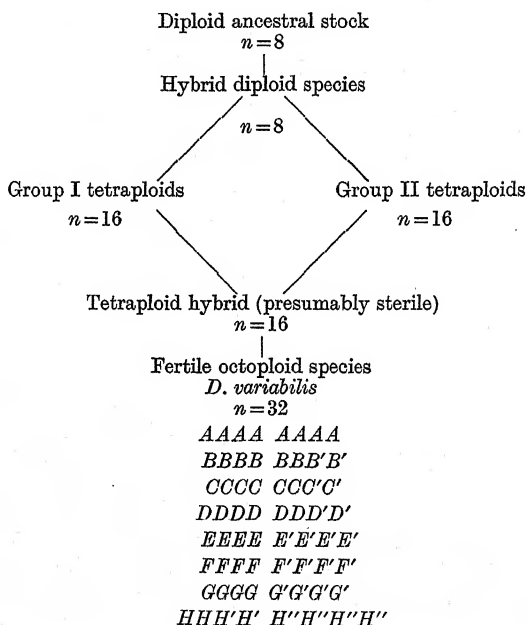
Text-fig. 9. The *G* chromosomes carry the *A* anthocyanin factors. The *G'* chromosomes carry the *B* anthocyanin factors. The *H* and *H'* chromosomes carry the *I* flavone factors. The *H''* chromosomes carry the *Y* flavone factors.

Y has differentiated from **I** or **I** from **Y**, or both originate from a common source.

It is highly probable that these are only two of several differences between the *G* and *G'* and *H* and *H''* chromosomes respectively, and among any of those chromosomes which form quadrivalents and sexivalents. The process of differentiation is continuous, and doubtless *D. variabilis* is not the same now as when it first arose. This is especially true of the *H*-chromosome type which, being heterozygous for **I** in one

pair of chromosomes, must have changed *after* the doubling of the chromosomes in the sterile hybrid.

The nature of the changes resulting in the four flower-pigment factors is suggestive. Assuming that the flavones are apigenin and luteolin, the essential portions of the structural formulae for the four pigments are given in Text-fig. 10. The anthocyanins are shown in their non-glucosidal form. As will be seen, the difference between apigenin and luteolin is exactly paralleled in the difference between pelargonidin and cyanidin. Structurally the change is a simple one, involving the loss or gain of one hydroxyl group.

If anthocyanins are produced by oxidation or reduction from flavones, the structural relations of the four pigments suggest that apigenin is the yellow flavone in *Dahlia*, as reported by Schmid and Waschkau.
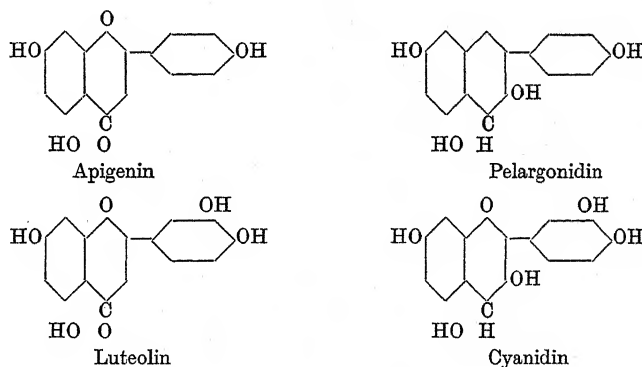
Text-fig. 10. Structural formulae for the non-glucosidal flavone and anthocyanin pigments of *Dahlia*, showing parallel differentiation between apigenin and pelargonidin, and luteolin and cyanidin respectively.

At present, however, the evidence is somewhat contrary to the assumption that such a direct and simple relation exists between the flavones and anthocyanins.

It is hoped that an analysis of the pigments in individuals of selected genetical constitution will be possible in the near future. The general indications are such as to suggest a relation between the yellow flavone and the deep anthocyanin. In the first place the **Y** and **B** colours are considerably deeper than the **I** and **A** colours respectively; and further the **Y** and **B** colours are both subject to bleaching, whereas the **I** and **A** colours so far have been found to be practically constant. Secondly, on the theory that the **I** and **A** factors were contributed by one parent of *variabilis* and the **Y** and **B** factor by the other, it is more probable that **Y** and **B** are related than **Y** and **A**.

Whether there is any close correlation from the chemical point of view cannot at present be determined, but the occurrence of **Y** and **B** and **I** and **A** in the separate colour groups of the species, as the products of differentiation from a common stock, suggests that, even though the respective flavones and anthocyanins may not be derived one from the other, their synthesis at least may be along common lines.

If the presence of pelargonin in purple flowers does not arise from a source extraneous to that of the four flower-colour factors, then it seems probable that the **A** factor will be found to produce cyanin, the **B** factor pelargonin, irrespective of the kind of flavone present.

Conversely, if the pelargonin in purple flowers is due to "contamination" from foliar pigmentation, the factors **A** and **B** must then each produce one pigment in the presence of ivory and another in the presence of yellow.

The experiments show that *Dahlia variabilis* combines the products of specific differentiation with a high degree of polyploidy—*i.e.* qualitative combined with quantitative differences. The result of this is seen in the extraordinary variation in colour, pattern, size, shape, habit, etc. found in the "variable" dahlia. This variation is infinitely greater than that of the tetraploid species, which breed relatively true. It is highly improbable that the potentialities of the garden dahlia to vary have been exhausted. Indeed the inherent self-incompatibility of *D. variabilis* tends to conserve any new character that may appear, and to keep the species in a heterozygous state bordering around the simplex or duplex condition.

SUMMARY.

Morphology.

1. *Dahlia* species, with the exception of *D. variabilis*, may be placed in one of two flower-colour groups: (1) ivory-deep magenta, or (2) yellow-orange-scarlet. Both colour series are found in *D. variabilis*.

2. Flower colour in *D. variabilis* is the expression of two series of soluble pigments: (1) flavones, (2) anthocyanins. Ivory and yellow form the flavone grounds upon which the anthocyanins are superposed.

Genetics.

3. Ivory ground is determined by the factor **I**, the inheritance of which is disomic.

4. Yellow ground is determined by the factor **Y**, the inheritance of which is tetrasomic.

5. Anthocyanin coloration is governed by two independent factors. **A** produces relatively pale pigmentation; **B** deep pigmentation. The inheritance of **A** is tetrasomic.

6. Mosaicism of the flower colours is confined to the anthocyanins. The pale anthocyanin is mosaic only in the presence of mosaic deep pigment. Self-coloured derivatives of mosaic individuals breed true for self-colour. The experiments on mosaic forms suggest that the inheritance of the mosaic factor is Mendelian.

Mutations.

7. Somatic mutation of anthocyanins and flavones is relatively common in the ray florets. The evidence strongly suggests that this is due to irregular distribution of the chromosomes in mitotic divisions.

Chemical.

8. The reactions of the flower colours are discussed. The flavones are probably apigenin and luteolin; the anthocyanins, cyanin and pelargonin.

Incompatibility.

9. *D. variabilis* is self-incompatible. Pseudo-fertility is stimulated by certain cross-incompatible pollinations. Cross-incompatibility was found in more than 25 per cent. of cross-pollinations. Cross-fertility varies continuously from 1–100 per cent. These data are discussed.

Cytology.

10. Four species examined, *D. coronata, D. coccinea, D. Maxoni* and *D. imperialis,* were found to be tetraploids with 32 chromosomes; a fifth, *D. Merckii,* has 36 chromosomes. *D. variabilis* is an octoploid with 64 chromosomes.

11. Terminalisation of the chiasmata is complete in *D. variabilis.* Only two configurations are found at meiosis. The first has a single terminal chiasma and terminal attachments to the spindle fibres. The second has two terminal chiasmata and median attachments. The combination of complete terminalisation and the attachments mentioned is shown to constitute a mechanism which will ensure great regularity of disjunction at meiosis of an auto-polyploid.

12. Two types of chromosome association are found in meiosis: (1) primary or prophase pairing, (2) secondary association which originates at pro-metaphase. Primary association in *Dahlia variabilis* results in quadrivalent and sexivalent associations. Secondary association results

Whether there is any close correlation from the chemical point of view cannot at present be determined, but the occurrence of **Y** and **B** and **I** and **A** in the separate colour groups of the species, as the products of differentiation from a common stock, suggests that, even though the respective flavones and anthocyanins may not be derived one from the other, their synthesis at least may be along common lines.

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12. Two types of chromosome association are found in meiosis: (1) primary or prophase pairing, (2) secondary association which originates at pro-metaphase. Primary association in *Dahlia variabilis* results in quadrivalent and sexivalent associations. Secondary association results

in groups of 4, 6 and 8 chromosomes. Secondary association is briefly discussed.

Interspecific hybrids.

13. Cross pollinations were made between *DD. Merckii* $(2n = 36)$, *coccinea* $(2n = 32)$, *coronata* $(2n = 32)$ and *variabilis* $(2n = 64)$. With one exception these all failed. Two hexaploid hybrids $(2n = 48)$ were raised from *variabilis* and *coronata*. The morphology of both hybrids and the cytology of one are described. Many of the *coronata* chromosomes pair with the *variabilis* homologues. Anomalous bivalent and multivalent configurations, such as are not found in *Dahlia* species, occur generally. These are shown to be the result of pairing between homologous but structurally differentiated chromosomes contributed by the respective parents. Though differing in chromosome number, *variabilis* and *coronata* are thus closely related.

Conclusions.

1. The results of the experiments are correlated and discussed. They strongly support the conclusion that *D. variabilis* is a hybrid octoploid derived from the crossing of two tetraploid species, one belonging to the ivory-deep magenta and the other to the yellow-orange-scarlet group. Doubling of the chromosome complement of this sterile, tetraploid hybrid gave rise to the fertile, double auto-tetraploid, *D. variabilis*.

2. The evidence suggests (1) that the factors **I** and **Y** for ivory and yellow flower colour have differentiated one from the other or from a common source, (2) that **I** and **Y** are carried by homologous chromosomes contributed by the group I and II progenitors respectively. The factorial and chemical differences between **I** and **Y** and the flavones they govern are an expression of the structural differentiation which prohibits pairing between the two homologous quadrivalent sets carrying **I** and **Y**. Similarly **A** and **B** are carried by two differentiated quadrivalent sets. The chemical differentiation of **I** and **Y** and of **A** and **B** respectively is probably parallel.

3. The results of the experiments suggest that the tetraploid *Dahlia* species have descended from a diploid ancestral stock. In their descent differentiation has occurred, giving rise among other differences to the two flower-colour groups. *D. variabilis*, therefore, combines the products of this specific differentiation with a high degree of polyploidy, *i.e.* qualitative combined with quantitative differences.

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EXPLANATION OF PLATE IX.

Figs. 1–4. Types of magenta-coloured rays with ivory grounds, from plants lacking **B** and **Y**. Figs. 1 and 2, violet-magenta; Figs. 3 and 4, rosy-magenta. The palest and deepest forms are shown in each case. (Fig. 1 is the colour of *D. Merckii.*)

Figs. 5 and 6. Types of apricot-coloured rays with yellow grounds, from plants of the constitution **AaaabbbbYyyyII.** On an ivory ground the anthocyanin would appear as in Figs. 1–4.

Figs. 7 and 8. Rays from the same plant. Fig. 7 shows the type. Fig. 8 shows a ray from a mutant branch in which the yellow ground has changed to a paler yellow, presumed to be due to the action of the yellow inhibitor.

Fig. 9. Purple ray showing mutant sector, due to the loss of the **B** factor in an individual of the constitution **AaaaBbbbyyyyII**. The pale pigmentation produced by the **A** factor is thus revealed.

Fig. 10. Four mutant sectors side by side, probably due to the effects of an irregular mitosis.

Fig. 11. Crimson petal in half of which the yellow ground has changed to ivory owing to the loss of the **Y** factor.

Figs. 12 and 13. Types of mosaic rays. Fig. 12 shows a fine mosaic deep pigment on a coarse mosaic of magenta. In this particular example the mosaic is so coarse that one half of the ray appears magenta, and the other ivory. Fig. 13 shows a coarse mosaic of deep pigment over self-coloured pale pigment.

Fig. 14. Ray of *D. variabilis* seedling 36/26 ($2n=64$).

Fig. 15. Ray of *D. coronata* ($2n=32$).

Fig. 16. Ray of the hybrid 2/29 (*i.e. D. variabilis* × *D. coronata*) ($2n=48$).

For the coloured drawings I am much indebted to Mr. H. C. Osterstock.

PLATE IX

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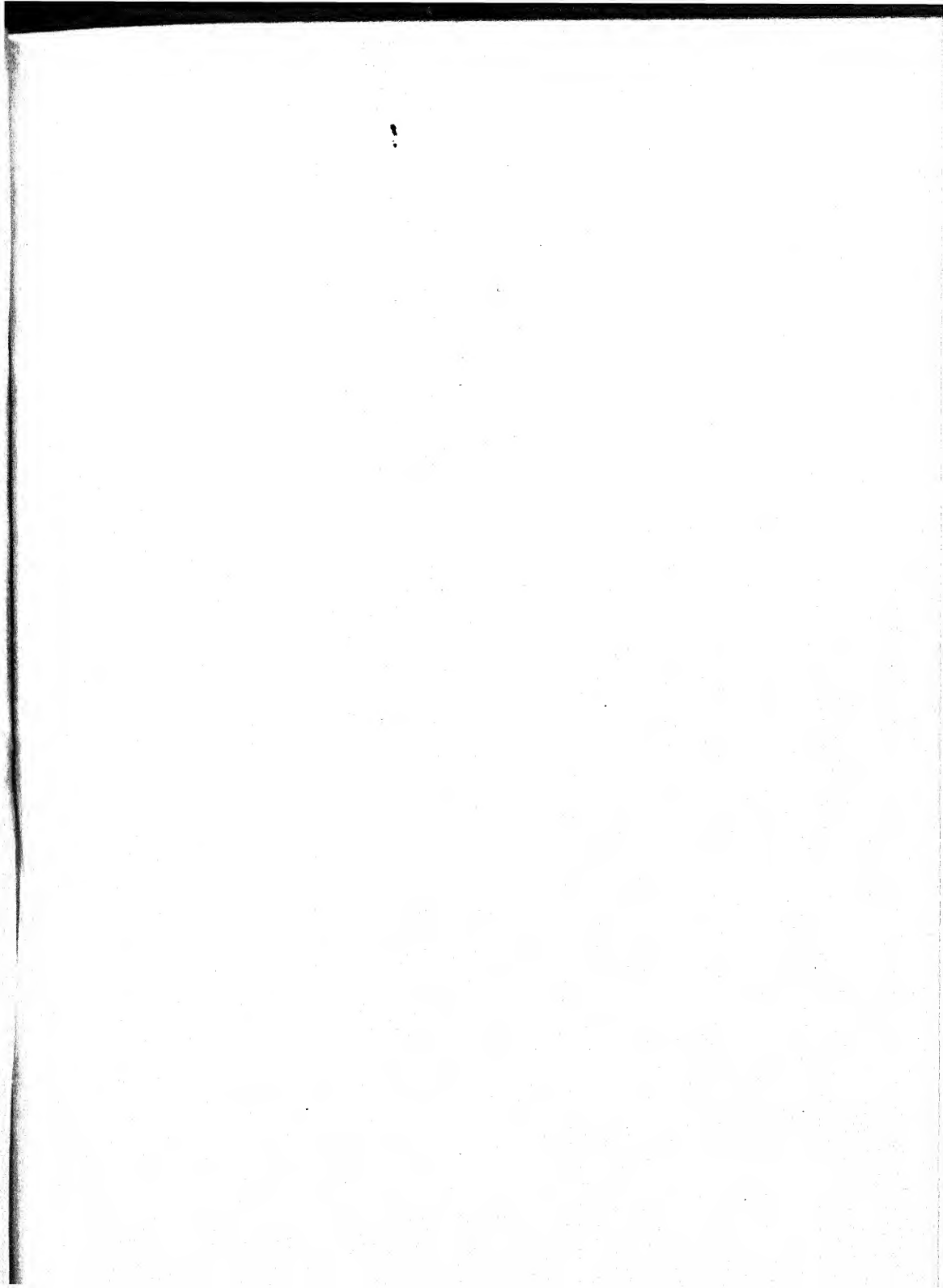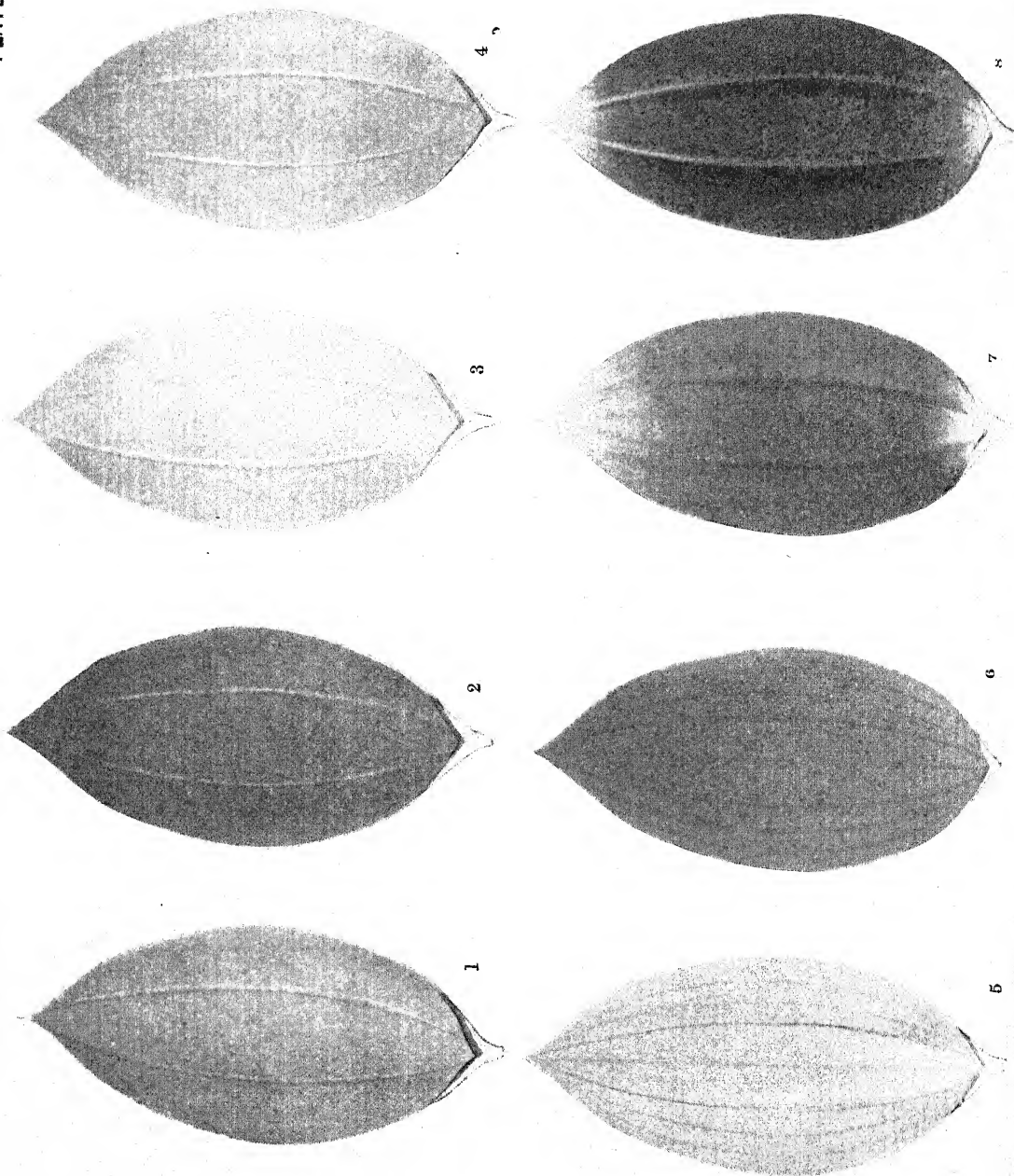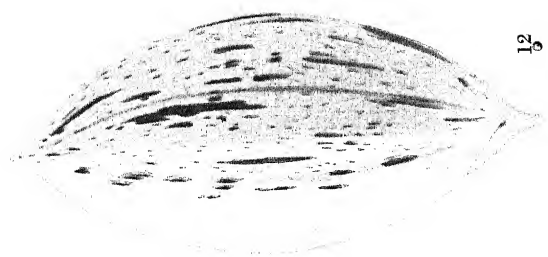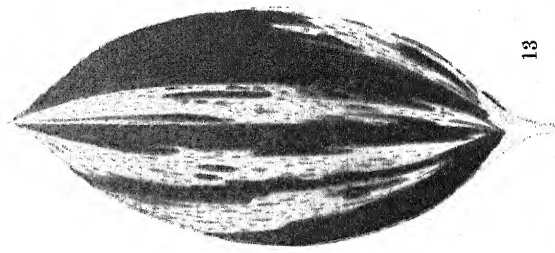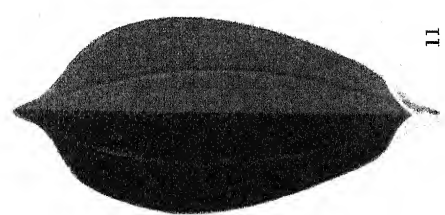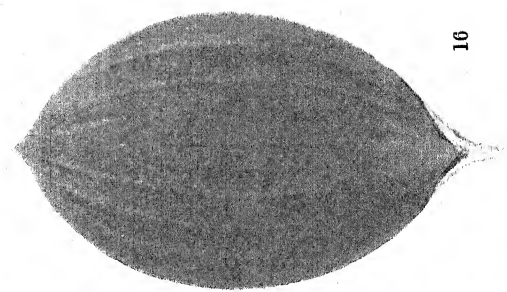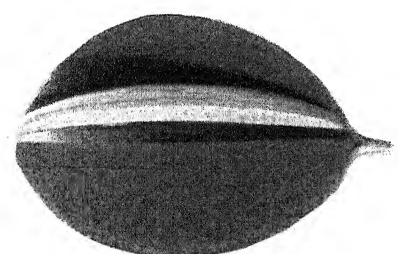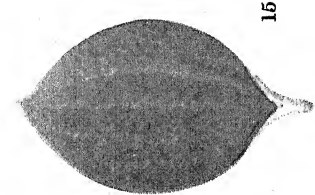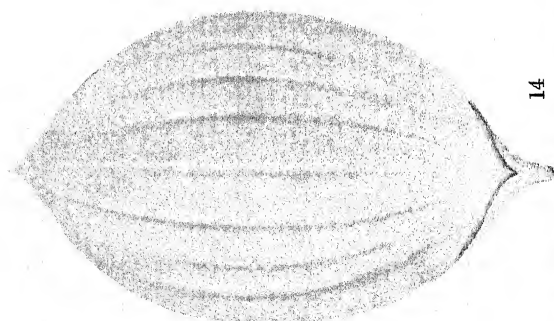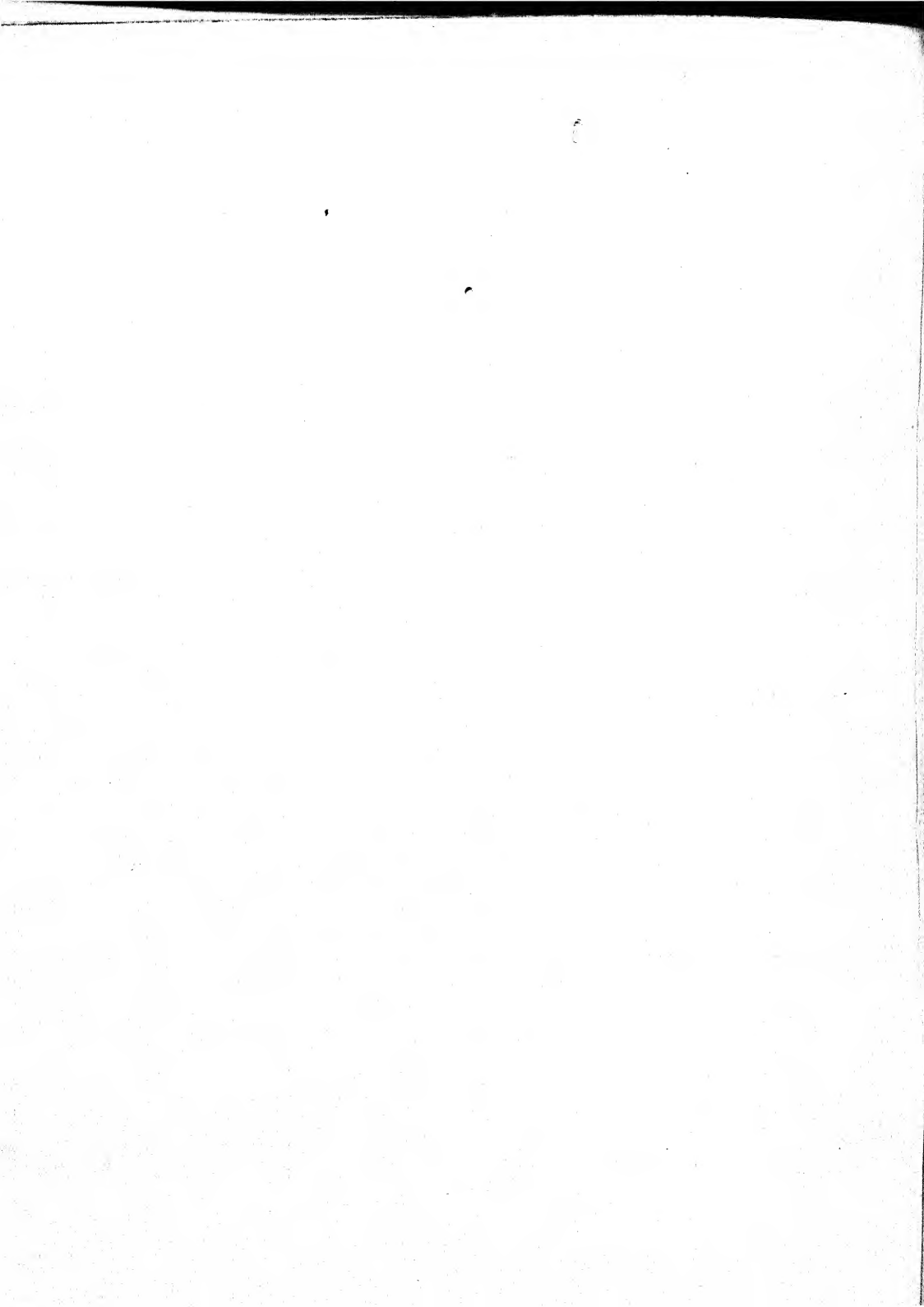
MUTATION OR SEGREGATION IN THE OCTOPLOID *DAHLIA VARIABILIS*.

By W. J. C. LAWRENCE.

(John Innes Horticultural Institute, Merton.)

(With Nine Text-figures.)

INTRODUCTION.

IN 1924 the late William Bateson was attracted by the unique flower-colour patterns shown by certain varieties of the garden dahlia (*D. variabilis*), the behaviour of which seemed to indicate a constitution chimerical for flower colour. The material was subsequently handed over to me for the study of the inheritance of the colour patterns. As the experiment progressed, however, it became apparent that *Dahlia variabilis* did not provide the best of material for such a study—for this species is octoploid (or, more correctly, a double auto-tetraploid). In addition the prevalence of incompatibility seriously frustrated schemes of breeding. The complex nature of the colour patterns and their unusual behaviour make interpretation of the observations and breeding results difficult. Nevertheless certain of them seem of sufficient interest to warrant description.

MATERIAL.

Ray floret colour in *Dahlia* is due to the presence or absence of two series of soluble pigments—(*a*) flavones, (*b*) anthocyanins. The flavones are responsible for the yellow and ivory ground colours upon which are superposed the anthocyanins. When both flavones and anthocyanins are absent the colour is white. Except in the pollen, flavone is present in the whole of the capitulum, and in purple-leaved forms anthocyanin occurs also, and is conspicuous in the disc florets.

The peculiar patterns investigated have a wide range of variation. The median condition is well represented in the variety Union Jack (syn. Helvetia) (see Fig. 1, end of second row) in which each ray floret is edged with crimson-scarlet, the remainder of the floret being a translucent white. This "abnormal-white" area often seems to be very faintly-tinged magenta owing to the reflection of light. The term "ab.-white" will be used to indicate the *whole range* of variation of this phenomenon, which

is due to the complete absence of all pigments in the affected portion of the capitulum.

In the rays the affected areas are a translucent white and, fumed with ammonia, give no reaction whatever. The normal white (*i.e.* the "bottom
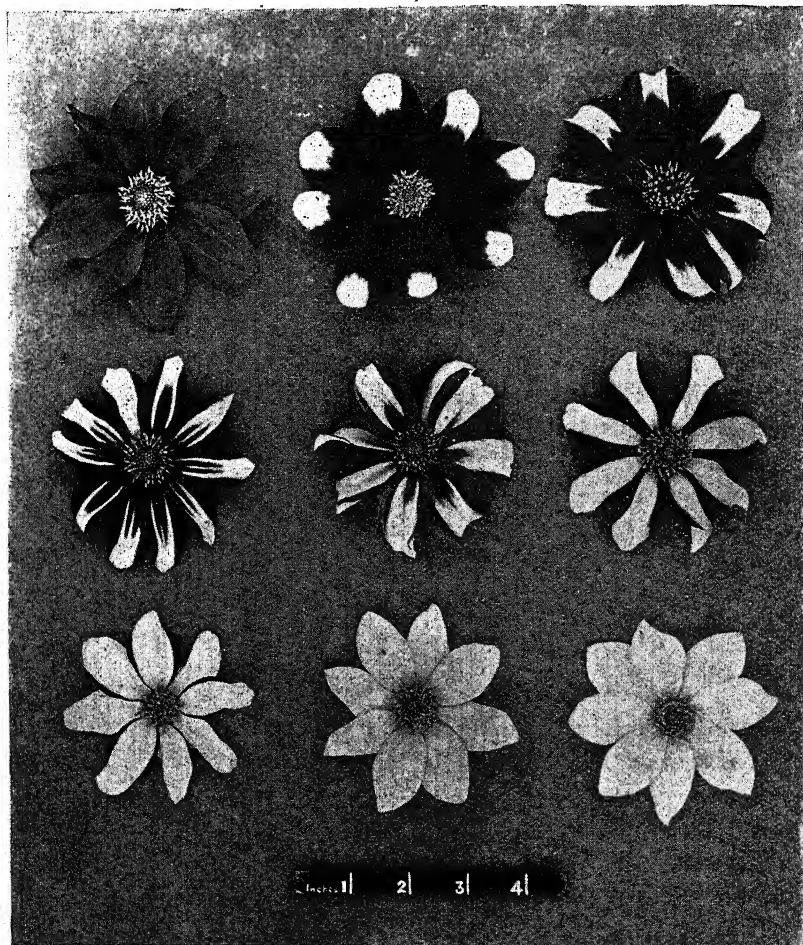
Fig. 1. Degrees of "ab.-white" as expressed in the ray florets of *D. variabilis*. Top left corner, a normal capitulum. Bottom right corner, an "all-ab.-white" capitulum. The remainder are "edged" forms.

recessive" condition) similarly fumed gives a negative result for the presence of flavones, but, unlike the ab.-white, becomes discoloured—a difference which is easily recognised. The ab.-white condition is accom-

panied by diminution in size of the affected part of the rays. It is most conspicuous in capitula which have normal and wholly ab.-white petals side by side. In such varieties as Union Jack this inequality of growth results in the wavy petal typical of the colour-edged forms. In the
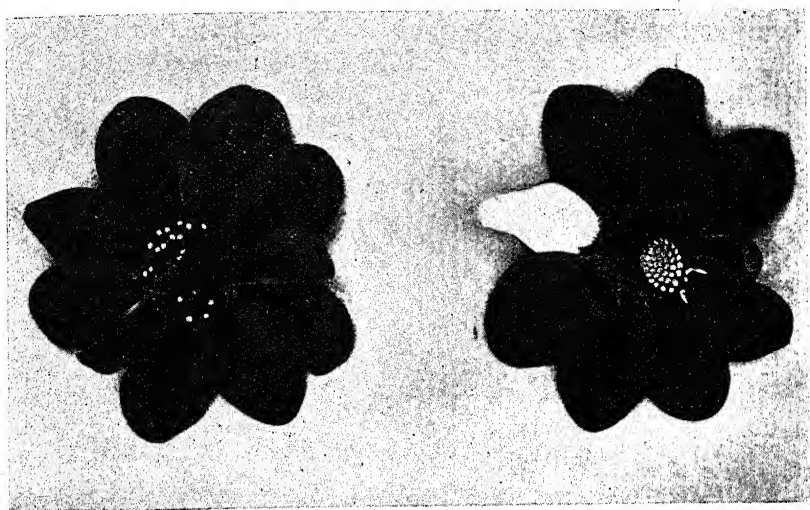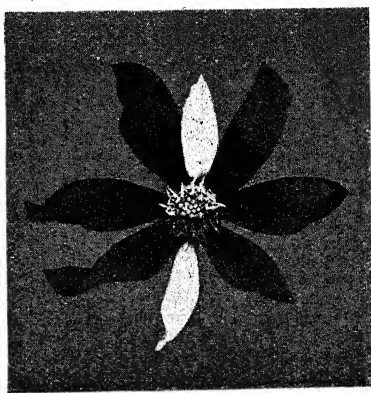
Fig. 2. "Ab.-white" as expressed in the disc florets: (a) pale florets occurring at random; (b) and (c) sectorial distribution. Each capitulum fumed with ammonia.

remainder of an affected capitulum the disc florets and bracteoles are noticeably paler than normal and, with the outer bracts and calyx, give a negative reaction when fumed. The proximal portion of the peduncle

may also be affected. In the normal white only the rays are devoid of flavone; the disc is fully coloured as in forms with normal-coloured rays.

The extreme form of ab.-white is that in which the whole of the capitulum is without flavones and anthocyanins. Since the expression of ab.-white is almost entirely confined to the capitula, there may arise the anomalous condition of pure white flowers on a plant with pigmented stems and foliage. Between this extreme and the normal every gradation is found. Fig. 1 shows some of the grades which occur in ray floret pattern. The disc of the capitulum may be mosaic for ab.-white, the
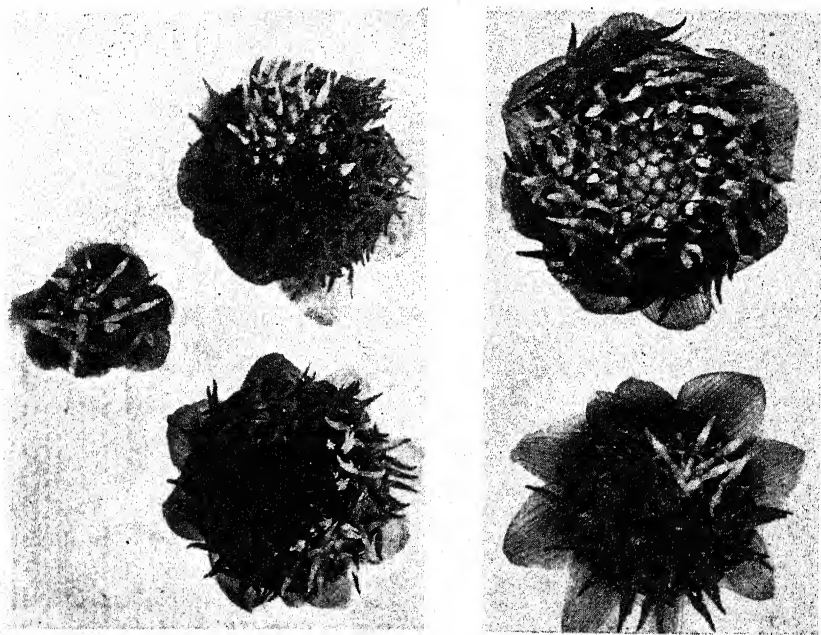
Fig. 3. Discs of "ab.-white" capitula after fuming with ammonia. Normal florets are dark coloured, abnormal florets are pale.

pale florets occurring indiscriminately, or sectorially, in which case they usually occupy the central portion of the disc (Figs. 2 and 3). Correlation between ray floret and disc pattern is evident. If all the rays show an ab.-white area (no matter how small), then the whole of the disc will be pale. *This is invariable.* But a pale disc need not be associated with ab.-white or colour-edged petals. Forms have been seen where the capitulum was normal save for a pale disc, or portion of a disc, and even one stigma only may be pale in an otherwise apparently normal flower.

A given plant may be completely or relatively stable in the production of flowers of a uniform pattern; and instability of high degree also is common. The only stable forms other than the normal varieties are the all-ab.-white forms, and very rarely, colour-edged varieties with a minimum of colour. Next to these, varieties like Union Jack whose rays have symmetrically coloured edges, though they may sport self-
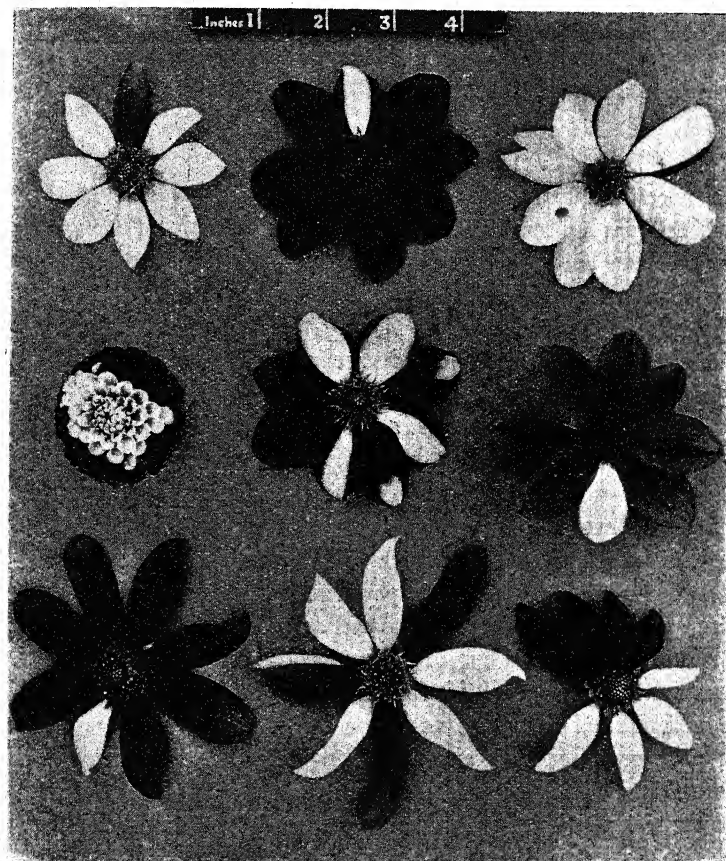
Fig. 4. Irregular distribution of "ab.-white" in various capitula.

coloured or white rays, vary but little in the degree of striping from capitulum to capitulum. Union Jack tends to produce more self-coloured flowers as it grows, and conversely other varieties produce fewer coloured flowers as growth proceeds. The least stable varieties are those with an irregular sectorial distribution of colour (as opposed to the sym-

metrically patterned types (see Fig. 4)). Nevertheless lack of stability is not necessarily associated with a sectorial distribution of colour, since other plants occur which are relatively stable for two kinds of flowers, *e.g.* (*a*) wholly self-coloured, and (*b*) all-ab.-white respectively, and these extremes may be produced without the appearance of intermediate types.

The branching of dahlia is dichotomous, and in plants grown from seed a primary capitulum appears while the plant is still young. A plant which later is to be ab.-white (in any degree) almost invariably produces a primary capitulum all-ab.-white or colour-edged—even if the subsequent growth bears apparently normal capitula.

BREEDING METHODS.

Unexpected difficulties were encountered in the breeding of ab.-white plants. All garden *Dahlia* varieties are self-incompatible, and cross-incompatibility has frequently upset prearranged schemes. Reciprocal crosses have often failed one way, and it is obvious that the closer the breeding the greater will be the incompatibility encountered. Increase in the degree of ab.-white is usually accompanied by loss of vigour, and large families are, in certain cases, difficult to obtain.

In dealing with so inconstant a pattern as ab.-white, it was essential that the least variable types should be used as parents. The normal plants chosen were, from their breeding and behaviour, known to be free from ab.-white. The all-ab.-white parents were used because it seemed improbable that their germ track would be coloured or mosaic. In the case of the colour-edged parents the discs of these are always uniformly pale, and this again seemed to rule out another inconstant factor. The self-coloured capitula from the ab.-white plants were always examined for the presence of pale disc florets and, wherever possible, only those with normal discs were used—or if one or two pale florets occurred these were removed before the anthers dehisced. The inconstancy of pattern made it necessary to score the plants several times during the flowering season. Each individual was scored four times at intervals of about two weeks on the following scheme:

Grade
0   Apparently normal
1   One or more pale disc florets (ray florets self-coloured as in normal)
2   Occasional ray florets ab.-white as well
3   Half the plant with ab.-white capitula
4   Three-quarters of the plant with ab.-white capitula
5   All the plant with ab.-white capitula

The four scorings were then averaged to give the degree of ab.-white. Since variation usually occurs to the next higher or lower grade, and rarely

to more than one grade away, the average figure obtained is a fairly reliable measure of the degree of ab.-whiteness for each plant. In a given family the percentage of plants showing ab.-white sometimes includes individuals which were scored in the 0 class owing to their average degree of ab.-white falling below 1.

Means for ab.-white were then calculated for (a) the whole family, and (b) the ab.-white plants only.

The degree of ab.-white in the parent capitula is indicated by the abbreviations normal, self, edged and a.a.w. (indicating normal, apparently normal, colour-edged and all-ab.-white respectively). The "self" class consists of apparently normal capitula from off ab.-white plants. The table is arranged so that all possible combinations are made—commencing with normal × normal coloured capitula from ab.-white plants, and proceeding to the other extreme. Eight classes are thus formed, the parent capitula of which show increase of ab.-white successively. The percentage totals enable comparison to be made between the groups.

BREEDING RESULTS.

Analysis of Table I and the results of previous work on colour inheritance (Lawrence, 1929 and 1931) reveal the following results.

(1) The ab.-white condition occurs and is inherited independently of the flavone and anthocyanin factors, whose inheritance is disomic or tetrasomic.

(2) In these experiments normal plants bred together have always given normal progeny. Grades 3, 4 and 5 have never occurred, and though small sectors of coloured rays have (rarely) sported white sectors it has not been possible to determine if they were ab.-white. Pale florets have never been observed.

(3) From Table I we see that whatever the appearance of the capitulum chosen as parent—if it came from an ab.-white plant—a proportion of ab.-white individuals will be found in F. Thus self-coloured capitula, not to be distinguished from the normal, when crossed to normals consistently produced a number of ab.-white progeny.

(4) Fig. 5 shows graphically the gross percentage of ab.-white plants and the proportion of class 5 in the progeny of the classes. Correlation exists between the *degree* of ab.-white in the parent *capitula* and the proportion of ab.-white *plants* in the progeny.

(5) Inspection of Table I shows that the bulk of the progeny falls into the end grades 0 and 5. The higher the degree of ab.-white in the

TABLE I. *Inheritance of abnormal-white.*

Reference no.	Family no.	♀ Type	♀ No.	♂ No.	♂ Type	Type Normal	Plants	% ab.-white	0	1	2	3	4	5	Mean of family	Mean of ab.-white plants
—	—	Normal	—	—	Normal	Normal	841	0·0	841	0	0	0	0	0	—	—
Normal × Self																
1	27/28	Normal	14/26	$22^8/27$	Self	Self	52	53·8	25	8	16	2	0	1	0·9	1·9
2	24/27	Ditto	Ditto	U.J.	Ditto	Ditto	43	20·9	35	1	2	3	1	1	0·5	2·8
3	38/28	Self	U.J.	14/26	Normal	Normal	24	45·8	16	3	0	1	2	2	1·0	3·0
4	39/28	Ditto	Ditto	Ditto	Ditto	Ditto	24	41·6	14	3	5	0	2	0	1·0	2·4
5	18, 26/27	Normal	34/26	U.J.	Self	Self	18	27·7	14	3	1	0	0	0	0·2	1·2
6	40/29	Ditto	Ditto	Ditto	Ditto	Ditto	45	42·2	27	10	1	1	4	2	0·9	2·2
7	39/29	Self	U.J.	34/26	Normal	Normal	64	40·6	41	7	6	5	1	4	0·8	2·5
8	22/29	Normal	$27^3/27$	U.J.	Self	Self	14	21·4	11	1	2	0	0	0	0·3	1·6
9	23/29	Ditto	$27^9/27$	Ditto	Ditto	Ditto	21	4·7	20	0	0	1	0	0	0·1	3·0
10	28/29	Self	U.J.	$27^9/27$	Normal	Normal	6	33·3	4	1	0	0	0	1	1·0	3·0
Total	—	—	—	—	—	—	311	332·0	207	37	33	13	10	11	6·7	23·6
Percentage	—	—	—	—	—	—	—	33·2	66·7	11·9	10·6	4·2	3·2	3·5	0·67	2·36
Normal × Edge																
11	23/27	Normal	14/26	U.J.	Edged	Edged	83	43·3	48	13	7	3	1	11	1·1	2·7
12	31/27	Edged	U.J.	34/26	Normal	Normal	60	48·0	32	6	8	2	3	9	1·4	3·0
13	25/27	Normal	14/26	$2^4/24$	Edged	Edged	17	64·7	8	2	3	1	2	1	1·4	2·6
Total	—	—	—	—	—	—	160	156·0	88	21	18	6	6	21	3·9	8·3
Percentage	—	—	—	—	—	—	—	52·0	55·0	13·1	11·2	3·7	3·7	13·1	1·3	2·7
Normal × A.a.w.																
14	37/29	A.a.w.	$22^9/27$	35/26	Normal	Normal	24	41·6	14	4	4	1	0	1	0·8	2·0
15	40/28	Normal	35/26	$22^9/27$	A.a.w.	A.a.w.	45	33·3	32	3	4	1	1	4	0·8	2·9
16	41/28	Ditto	32/26	$31^5/27$	Ditto	Ditto	31	38·7	19	3	1	1	0	7	1·4	3·6
17	41/29	Ditto	Ditto	Ditto	Ditto	Ditto	22	68·1	8	3	3	1	0	7	2·1	3·3
18	42/29	Ditto	14/26	$22^8/27$	Ditto	Ditto	16	75·0	7	6	2	1	0	0	0·8	1·4
19	26/28	Ditto	Ditto	Ditto	Ditto	Ditto	12	41·6	9	2	1	0	0	0	0·3	1·3
Total	—	—	—	—	—	—	150	298·3	89	21	15	5	1	19	6·2	14·5
Percentage	—	—	—	—	—	—	—	49·7	57·7	13·6	9·7	3·2	0·6	12·3	1·0	2·4

No.	Family	Parent 1		Parent 2											
Self × Self															
20	56/28	1²/25	Self	U.J.	Self	43	65·1	16	6	4	2	2	13	2·1	3·4
21	34, 35/27	Ditto	Ditto	Ditto	Ditto	68	47·0	36	7	11	4	2	8	1·3	2·7
22	37/27	Ditto	Ditto	27/24	Ditto	55	60·0	24	11	9	2	2	7	1·4	2·5
23	33/29	Glenshee	Ditto	U.J.	Ditto	76	77·6	20	7	11	16	4	18	2·4	3·2
24	31/29	Ditto	Ditto	Ditto	Ditto	69	72·4	21	7	9	6	6	20	2·4	3·4
25	30/29	Ditto	Ditto	Ditto	Ditto	53	64·1	22	5	6	9	7	4	1·7	2·7
Total	—	—	—	—	—	364	386·2	139	43	50	39	23	70	11·3	17·9
Percentage						—	64·3	38·0	11·7	13·7	10·7	6·3	19·1	1·9	2·99
Self × Edge															
26	36/29	1²/25	Edged	U.J.	Self	48	47·9	29	6	3	4	3	3	1·0	2·6
27	29/27	U.J.	Self	1²/25	Edged	36	55·5	18	2	5	0	3	8	1·7	3·5
28	29/29	Glenshee	Ditto	U.J.	Ditto	51	60·7	23	3	7	6	5	7	1·7	3·2
29	22/27	Ditto	Edged	U.J.	Self	66	59·0	37	10	14	1	1	3	0·9	2·0
30	34/29	U.J.	Ditto	Glenshee	Ditto	18	55·5	8	1	2	2	1	4	1·9	3·5
Total	—	—	—	—	—	219	278·6	115	22	31	13	13	25	7·2	14·8
Percentage						—	55·7	52·5	10·0	17·1	6·8	6·8	11·4	1·4	2·96
Self × A.a.w.															
31	43/29	Barilét	Self	22⁹/27	A.a.w.	47	57·4	22	1	0	0	2	22	2·5	4·7
32	38/29	22⁹/27	A.a.w.	Barilét	Self	57	49·1	32	4	3	1	3	14	1·6	3·8
Total	—	—	—	—	—	104	106·5	54	5	3	1	5	36	4·1	8·5
Percentage						—	53·2	52·0	4·8	2·9	0·9	4·8	34·5	2·0	4·2
Edge × Edge															
33	35/29	U.J.	Edged	Glenshee	Edged	7	71·4	1	0	1	1	1	3	4·5	4·1
34	—	U.J.	Self-pollinated			19	63·1	7	4	1	1	0	5	1·9	3·0
35	—	27/24	Self-pollinated			12	66·6	4	0	0	3	2	3	2·6	4·0
Total	—	—	—	—	—	38	201·1	12	4	2	6	3	11	8·0	11·1
Percentage						—	67·0	31·6	9·5	5·2	15·7	7·9	29·9	2·6	3·7
Edge × A.a.w.															
36	32/29	Glenshee	A.a.w.	U.J.	Edged	8	75·0	2	0	0	1	2	3	3·2	4·6
Percentage						—	—	25·0	—	—	12·5	25·0	37·5	3·2	4·6
2²/24 crosses															
37	14/28	14/26	Normal	2²/24	A.a.w.	44	100·0	1	3	4	1	2	33	4·2	4·3
38	28/27	2³/24	A.a.w.	14/26	Normal	26	88·4	4	2	1	1	0	18	3·7	4·4
39	33/27	27/24	Edged	2²/24	A.a.w.	36	91·6	2	2	1	4	0	27	4·2	4·4
Total	—	—	—	—	—	106	280·0	7	7	6	6	2	78	12·1	13·1
Percentage						—	93·3	6·6	6·6	5·6	5·6	1·9	74·5	4·0	4·3
Grand total	—	—	—	—	—	1460	—	713	160	158	90	65	274	62·7	116·4
Percentage						—	—	48·5	10·9	10·8	6·1	4·4	18·7	1·6	3·0

parent capitula, the greater the proportion of grade 5 individuals in the progeny. An approximately corresponding decrease is found in grade 0.

It appears highly probable therefore that the frequency of ab.-white individuals in F_1 is proportional to the degree of ab.-white in the germ-track of the parental capitula. Those families with the greatest proportion of ab.-white individuals also reveal the highest degree of ab.-white per capitulum. This is best seen when the progeny of narrow- and broad-edged capitula are compared. Reliable analysis of this kind can only be
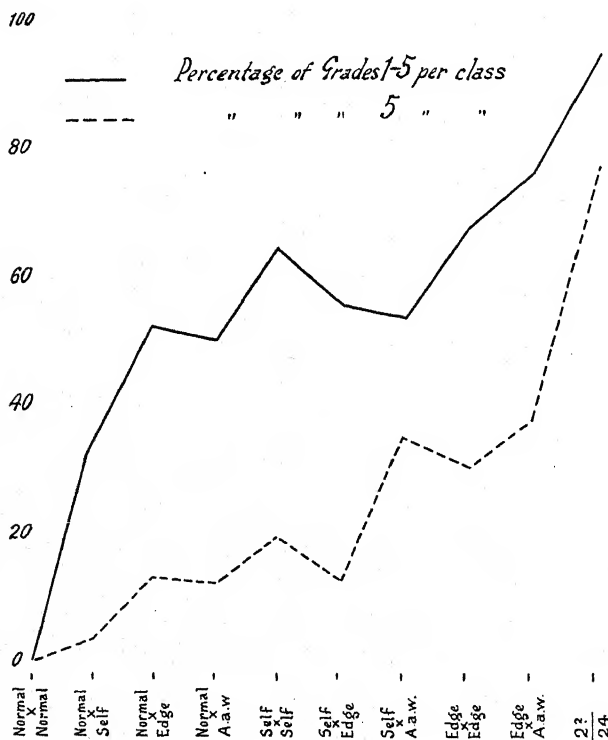
Fig. 5.

made on families which are relatively stable—but experience has shown that even where it is not practicable to measure this variation it nevertheless occurs.

(6) Both the means for degree of ab.-white in the classes and degree of ab.-white in the ab.-white individuals of the same classes show parallel increase proportional to the degree of ab.-white in parental capitula (Fig. 6).

(7) In Fig. 7 the percentage of the intermediate grades 1–4 is given. It is clear from this graph that the percentage of these grades is approximately constant in every class. Fig. 8 shows the gross percentage of ab.-white plants per grade. Grade 1 should probably be greater than
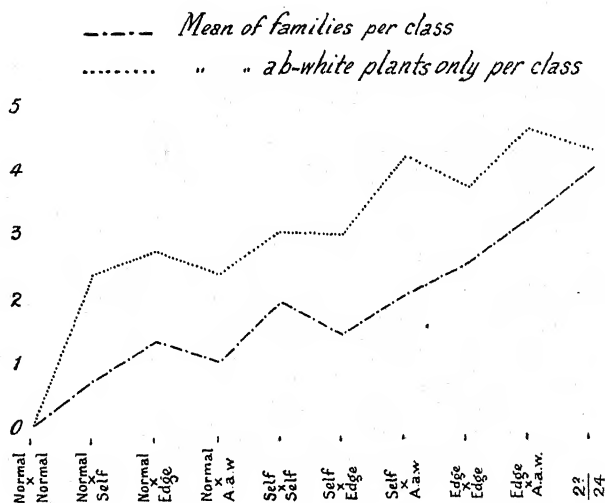
Fig. 6.
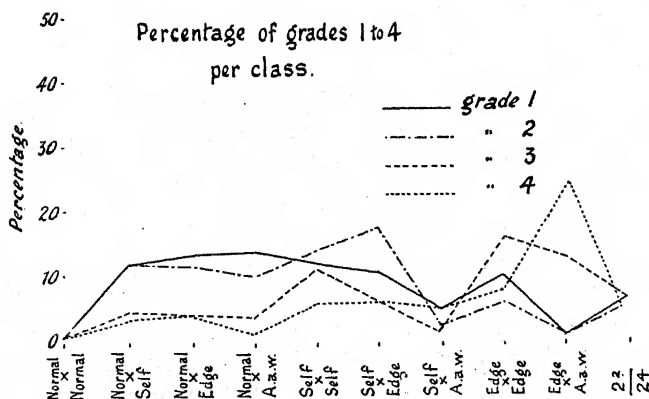
Fig. 7.

10·9 per cent. of the whole, for whereas grades 2–4 can be recognised instantly, grade 1 can only be scored after close scrutiny of the capitula, and failure to notice pale florets would be more likely to occur. A distinct decrease is seen in the direction of grades 1 to 4.
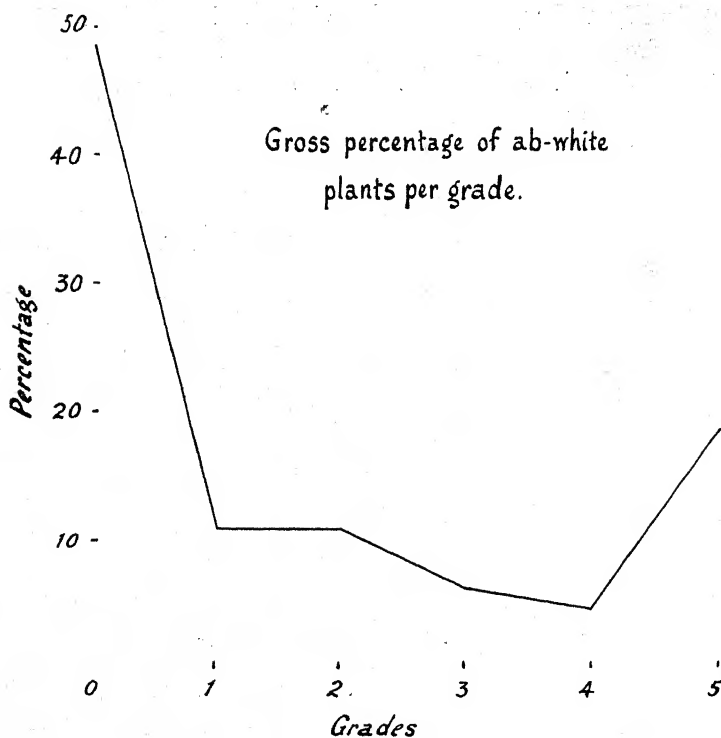
Fig. 8.

(8) Table II gives the proportion of grades 0, 1–4 and 5 in reciprocal crosses of normal and ab.-white plants. The figures are not large, but the differences are probably significant ($P = 0.02$–0.05). Grades 1 to 4 are

TABLE II.

Segregation in reciprocal crosses of normal and ab.-white.

	Normal × Ab.-white					Ab.-white × Normal			
Ref. nos. in Table I	Plants	Grade 0	Grades 1–4	Grade 5		Ref. nos. in Table I	Plants	Grade 0	Grades 1–4	Grade 5
2	43	35	7	1		3 and 4	48	30	16	2
5 and 6	63	41	20	2		7	64	41	19	4
9	21	20	1	0		10	6	4	1	1
15	45	32	9	4		14	24	14	9	1
37	44	1	10	33		38	26	4	4	18
Total	216	129	47	40		Total	168	93	49	26
Percentage	—	59·7	21·7	18·5		Percentage	—	55·3	29·1	15·5

increased by more than 7 per cent. at the expense of grades 0 and 5 when the female parent is ab.-white.

(9) Table III gives the results from breeding apparently normal derivatives from ab.-white crosses. An F_1 was raised from family 31/27

TABLE III.

Family	Class and cross	No. of plants	% ab.-white	Plants per grade			% grade 5
				0	1–4	5	
31/27	Edge × Normal	60	48	32	19	9	15
41/28	Normal × 31⁵/27 (all-ab.-white)	53	49	27	12	14	26
—	41¹/28 (Norm.?) × 41²/28 (Norm.?)	52	6	49	3	0	—
—	41¹/28 (Norm.?) × Normal	25	0	25	0	0	—
—	Normal × 41¹/28	93	2	91	2	0	—
	Total	170	—	165	5	0	—
28/30	Normal × 6²/29 (41¹ × 41² Norm.?)	6	—	5	1	0	—

by outcrossing an all-ab.-white flower from a grade 3 plant, 31⁵/27, to a normal. The increase of ab.-white in the F_1 is striking. From this F_1 two apparently normal plants 41¹/28 and 41²/28 were crossed together, with the result shown. 41¹/28 was also outcrossed reciprocally to a normal. Finally a small F_3 (28/30) was obtained by crossing an apparently normal F_2 individual 6²/29 with a normal plant.

In both F_2 and F_3 no grades 1, 3, 4 or 5 occurred in a total of 176 plants, and of the six individuals in grade 2 each had only one petal showing the ab.-white in the "edged" pattern.

We see then that normal derivatives of ab.-white crosses breed practically true for normality.

There seems to be little doubt that grades 1–4 are "mutants" from grades 0 and/or 5. If the colour change occurs early in the life of the individual, then a coarse "mosaic" of self-coloured and ab.-white branches will ensue; or if the change occurs very early, the whole plant may be uniformly coloured. The later the variation the smaller will be the area affected.

The difference in total numbers of grades 1–4 in reciprocal crosses of normal and ab.-white plants indicates increase in the rate of variation when the female parent is ab.-white.

The behaviour of the ab.-white pattern often suggests a chimerical distribution of colour. Thus the colour-edged forms are distinctly more stable than sectorial forms in which few or many relatively small areas are abnormal, but plants sectorial in large areas are usually constant for the respective areas.

Attempts were made to propagate cuttings and divisions of normal and ab.-white portions of given plants, and although the results were not conclusive it was obvious that selection of material for vegetative pro-

pagation could at least temporarily alter the grade of the variety. It is probable that continued selection of material could, apart from mutation, permanently alter the grade of the variety, thus establishing a new form relative to the original stock.

The fluctuation in grade as the flowering season advances is closely connected with the growth and development of the normal and ab.-white portions of the plant. If a somatic change in pattern should occur shortly after the plant commences to flower, then, as the mutated portion develops and gains the ascendancy over the earlier flowering branches, a progressive change in the grade will be observed. This change in grade will be dependent upon the direction of the variation, whether from ab.-white to normal or *vice versa*. Fluctuation in grade on the other hand will be dependent upon (1) the point where variation occurs in the development of the plant, and (2) the number and disposition of the branches involved. Since lateral branches in *Dahlia* develop successively, are terminated by an inflorescence and have a relatively short life, fluctuation in grade is a matter of balance between the flowering periods of the normal and ab.-white portions.

It is possibly significant that most fluctuations are towards the normal. As previously stated, the primary capitula on a plant carrying ab.-white are usually colour-edged or all-ab.-white, as if the production of pigments had run out in the branch which had nearly completed its growth—the amount of coloration depending upon the point of cessation of production of pigment. Indeed all the colour-edged forms are suggestive of this phenomenon which is expressed only in the vicinity of the inflorescence, for ab.-white individuals are normal in all other respects.

Comparison may be made here with two other members of the Compositae which have a somewhat similar pattern. The first, *Tagetes*, Star of India, has been fully described by Chittenden (1927). The ray florets of this plant have a brown margin and central yellow stripe. Considerable variation in the marginal area is found in every plant but, unlike *Dahlia*, capitula with varying amounts of colour (*i.e.* brown) give similar results upon selfing. There is a distinct diminution of brown pigment as the season advances. The second case I observed in a plant of *Calendula officinalis*, the rays of which had fairly regular orange edges and yellow central stripes (see Fig. 9). This one plant was quite stable but unfortunately was not discovered until too late to gather seed. A third case, also described by Chittenden, is *Myosotis*, Star of Zurich— characterised by the fact that there is a broad central white stripe on

each petal, the marginal area of every petal being blue. This variety gives only white-flowered progeny, and thus seems to be a blue-over-white periclinal. None of these cases, however, is strictly comparable with that of *Dahlia*, for in each of them the pattern is confined to the "petals."

Ab.-white therefore is a pattern which, unlike the great majority of colour-patterns, is not confined to the petals but is expressed in varying degree in the terminal portions of the flowering system.

Although five other *Dahlia* species are in cultivation, *variabilis* is the only one in which ab.-white is found. As I have shown (Lawrence, 1931), there is substantial evidence supporting the view that the garden *Dahlia* is an octoploid which has arisen from the crossing of two tetraploid
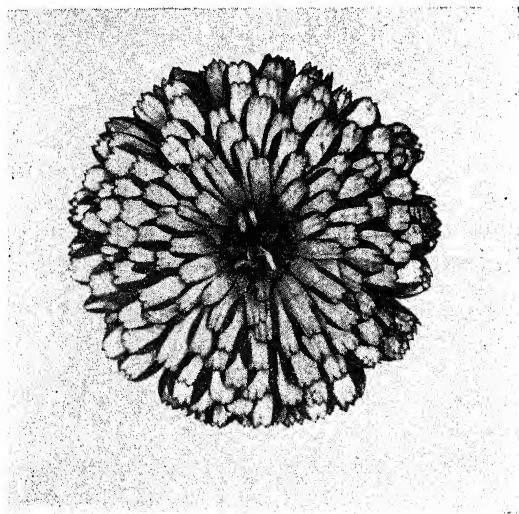
Fig. 9. *Calendula officinalis*. Ray petals yellow with orange edges.

species followed by doubling of the chromosome complement. Breeding work on flower colours has shown that segregation of the chromosomes is remarkably regular, and, though cytological observations indicate the possibility of hexasomic segregation of factors, the preponderance of quadrivalents at metaphase of the first meiotic division shows that segregation will be mainly tetrasomic.

The pattern "ab.-white" conforms to no other distribution of colour in *Dahlia*, neither does it even remotely approach the types of inheritance at present elucidated. The somatic variation and probable differences in reciprocal crosses are suggestive of a mode of inheritance far less regular than any based on segregation of chromosomes.

In this connection it must be remembered that half of the chromosome complement of *D. variabilis* functions in a foreign cytoplasmic environment, and thus a potential cause of abnormality is ever present. It is also significant that although the flavones and anthocyanins are carried by different chromosome types, yet *all* pigments are absent in ab.-white areas. Furthermore, other substances are similarly missing, for ab.-white gives a completely negative reaction when fumed with ammonia and so differs, as previously explained, from normal white. Again, the normal absence of pigments in a white-flowered variety does not cause diminution of vigour or size of the ray florets, yet considerable decrease of both is always evident in ab.-white areas.

Evidence as to the direction of variation is difficult to obtain. It is probable that it is mainly in the direction of abnormality, but may occur in both directions. The earliest record of an ab.-white plant known to the writer is found in *Harrison's Floricultural Cabinet*, vol. I—wherein the correspondent refers to Lewick's Incomparable grown (and raised?) in 1831.

The crimson rays of this double-flowered variety were tipped with white in almost exactly the same way as the second capitulum illustrated in Fig. 1. Two other varieties are recorded in 1836 as having white-tipped flowers, namely Viscountess Beresford and Beauty of Hammersmith. Coloured plates of Incomparable and Viscountess Beresford are shown in Smith's *Florists' Flowers* (London, 1836).

It seems probable therefore that the first ab.-white varieties arose somewhere about 1830, and since they must have arisen from normal plants it is more likely that the variation is generally in the direction of abnormality.

This view is supported by the proportions of grades 1–4, since the decrease in number in this direction coincides with expectation when the increase in the rate of cell-divisions during the growth of the plant is considered in relation to a more or less constant rate of variation. On the other hand, the decrease in number of grades 0 and 5 (as shown in Table II) when the female parent is ab.-white may indicate that variation is in both directions.

Referring to Table I, crosses 20 and 21, we see that totally different results were obtained from two crosses where similarly patterned capitula were used, and again in crosses 23, 24 and 25 the proportions of the grades in the first two are quite different from the third. It is noteworthy that the self-coloured capitula from ab.-white plants often give more ab.-white progeny than capitula with a higher degree of abnormality.

This is also exemplified in the reciprocal crosses, 26 and 27, and 28 and 29, where the use of self-coloured capitula as females gives a much greater proportion of class 5 than in the reciprocal cross. Evidently the distribution of pigment in self-coloured capitula is sometimes superficial in relation to the germinal tissue, and affords no real criterion of the genetic constitution of the germ cells. This is especially true in regard to self-coloured capitula of Glenshee—as it is a rare thing to find one apparently perfectly normal—*e.g.* a few pale florets frequently occur.

The variety Amy Barilét may be mentioned here. This plant was for several years regarded as a normal and then it produced one all-ab.-white petal. No other indication of abnormality had been observed in four years, yet its breeding behaviour is typical of the self-coloured class (see nos. 31 and 32, Table I). A solitary seedling, believed to have had an apogamous origin, obtained from selfing Amy Barilét, also produced a single ab.-white petal.

The polyploid constitution of *D. variabilis* is a complicating factor in its bearing upon the inheritance of ab.-white. It is quite clear nevertheless that in almost every respect inheritance is purely quantitative—the width of the coloured edge, the degree of abnormality and the proportion of class 5 per family.

What hypothesis can be found to fit the facts?

Labile genes might be supposed to be the source of variation but for the fact we must assume that their many different conditions are of different potency too—and that this potency is inherited. The theory of plastid inheritance is difficult to reconcile with the biparental inheritance of the pattern. Eyster's (1924) hypothesis of the compound gene does not allow for reciprocal differences and moreover is difficult to test.

It is more probable that we have interaction of genes and cytoplasm, and it would be best perhaps to attempt a solution to the problem in the following general terms:

(1) There are at least several units for inhibition of flower colour pigments.

(2) Both male and female parents make effective contributions to their progeny.

(3) Somatic distribution of the units is variable—the germ cells varying in constitution according to their place of origin in the soma.

(4) The pattern ab.-white is probably the result of the interaction of nuclear and extra-nuclear conditions.

SUMMARY.

A peculiar flower colour pattern in the octoploid *Dahlia variabilis* ($2n = 64$) is described. This pattern, "ab.-white," is due to the complete absence of all pigments from part or all of the capitulum. Ab.-white plants are unstable, and somatic variation is frequent.

Breeding experiments have shown that:

(*a*) Inheritance of the pattern is purely quantitative—the degree of ab.-white in the parent capitula determining the proportion of ab.-white progeny in the next generation.

(*b*) Both parents make effective contributions to their progeny, but the rate of mutation is apparently increased when the female parent is abnormal.

(*c*) The progeny from crossing ab.-white plants fall into two main groups—normal and completely ab.-white. Intermediate grades are shown to be due to somatic variation at different points in the growth of the plants.

(*d*) Normal derivatives of abnormal parents breed approximately true for normality.

(*e*) Vegetative propagation of selected shoots can temporarily alter the grade of ab.-white.

(*f*) The pattern is only found in the hybrid octoploid *Dahlia variabilis*.

(*g*) It is suggested that the pattern is the result of interaction of nuclear and extra-nuclear conditions.

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THE GENETICS OF COTTON. PART IV. THE INHERITANCE OF COROLLA COLOUR AND PETAL SIZE IN ASIATIC COTTONS.

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(With Plate X.)

CONTENTS.

INTRODUCTION.

THE data here presented are the result of the second part of an investigation into the inheritance of corolla colour and associated characters in *Gossypium*. The first part, dealing with the New World species of *Gossypium*, was published by Harland (1929). The present paper is concerned with the Asiatic Gossypiums, among which corolla colour ranges from deep yellow to ivory white, forming a colour series very similar to that described by Harland (1929) for the New World Gossypiums. Previous work in India indicated that there was an association between corolla colour and petal size, and corolla colour and lint length and ginning out-turn. These characters were therefore included in the investigation wherever possible.

PREVIOUS WORK.

Previous work on corolla colour has been reviewed by Harland (1929) and will only be dealt with briefly.

Fyson (1908) in crosses between yellow-flowered and white-flowered varieties obtained yellow F_1's, and yellows, pales, and whites in varying proportions in F_2. Some families gave a ratio of nearly 3 coloured to 1 white.

Fyson observed a strong correlation between petal size and corolla colour. In the progeny of a cross between a large-flowered yellow and a small-flowered white he obtained large-flowered yellows and small-flowered whites with a very small proportion of large-flowered whites, and no small-flowered yellows.

Leake (1911), and Leake and Ram Prasad (1914) found full yellow dominant in crosses of full yellow × pale and full yellow × white. In F_2 full × pale gave 3 full : 1 pale, and full × white gave 3 full : 1 white.

Leake (1911) confirmed Fyson's observations on the correlation of petal size and corolla colour, but stated that the correlation was complete, yellow-flowered plants always having large flowers, and whites, small flowers.

Kottur (1923) gave data from the F_2 and F_3 of a cross between yellow and white. An interpretation was suggested by Harland (*loc. cit.*). F_1 was recorded as pale, and in F_2 pales, full yellows and whites occurred in proportions close to 39 pale : 9 full : 16 white. Harland comments: "The ratio is probably a modified trihybrid one, the three factors concerned being:

A—a factor converting white (**a**) to pale yellow.
B—an intensification factor converting pale yellow (**A**) to full yellow.
C—a factor inhibiting the action of **B**."

Then the yellow-flowered parent must be **ABc**, and the white-flowered parent **abC**. In F_2 16 out of 64 lack **A**, and are therefore whites, 9 carry **A** and **B** but lack **C** and are full yellows, and the remaining 39 are pales of various constitutions. Harland demonstrated also a reasonable fit between observation and expectation among F_3 families[1].

Kottur (1923) confirms the existence of a strong correlation between colour and petal length. Frequency arrays are given for parents, which were long yellow and short white, F_1 and F_2, and for yellow-flowered and white-flowered F_2 plants separately. Long whites occur in very small numbers. Short yellows are very common.

Three types used in the present investigation—viz. Cawnpore Yellow, Cernuum, and Cawnpore White—were obtained from Leake and

[1] Dr Harland wishes to correct an error which has crept into his paper at this point. For **Y** and **Z** read **B** and **C** respectively.

Ram Prasad, and the main grouping into "yellows," "pales," and "whites" was based on their types.

Kottur states that his "pale" varies from 5·0 to 5·75 yellow on Lovibond's tintometer. It has not been possible to compare Plate X with Lovibond's scale, but it seems probable that Kottur's pale corresponds to the lighter shades of the yellows, say grades 4–6. The constitution of Kottur's "pale" will be further considered later (see "Discussion").

Perkin (1899, 1909 and 1916) studied the chemistry of the colouring matter of the yellow flowers of *G. herbaceum* and *G. neglectum*, and the white flowers of *G. roseum*, among the Asiatic Gossypiums, and of the yellow flowers of Egyptian among the New World Gossypiums.

Three glucosides were isolated from Egyptian flowers—quercimeritrin, isoquercitrin, and gossypitrin. Of these, isoquercitrin and gossypitrin were isolated from *G. herbaceum* and *G. neglectum*, and the presence of quercimeritrin in small quantities was suspected. Practically no colouring matter could be extracted from flowers of *G. roseum*.

SUMMARY OF PREVIOUS WORK AND STATEMENT OF AIMS.

Leake and Ram Prasad have demonstrated single-factor differences between yellow and pale, and between yellow and white, and Kottur has confirmed the existence of a single-factor difference between yellow and white. Kottur has observed a pale dominant to full yellow.

Fyson, Leake, and Kottur have all demonstrated a very close correlation between petal size and corolla colour.

In the present paper evidence will be presented to show:

1. That yellow, Leake's pale, and white form a multiple allelomorph series, which will be designated **Y, Yp**, and **y**.

2. That the correlation between petal size and corolla colour holds good for minor factors which affect corolla colour as well as for the main multiple allelomorph series.

3. That plants with yellow flowers and short petals, and white flowers and long petals occur as a result of the shuffling of these modifying factors and not as a result of crossing-over, and that these yellow short-petal types are lighter in colour than yellow long-petal types, and are similar to Kottur's pale.

4. That there is a physiological relationship between colour and size.

Evidence will also be presented to show that both main and minor corolla-colour factors affect lint characters as well as petal size.

METHODS.

The three main classes of corolla colour—yellow, pale[1], and white—are easily distinguishable. There is a considerable range of variation among yellows, and some variation among pales. No variation has been observed among whites, except on a few plants with very small petals. While there was no difficulty in classifying into the three main classes, it was necessary to have a scale of colours to grade against when studying the effect of modifying factors. A series of ten grades was painted and is reproduced in Plate X. Grades 4–10 are yellow. A very few yellow-flowered plants give flowers of grade 3. Grades 2 and 3 are pale. In certain heterozygotes between pale and white, plants occur with corolla colour between grades 1 and 2. With practice these are clearly distinguishable from grade 1. It was not possible to paint a grade that would fall accurately between grades 1 and 2, and these intermediate flowers were always graded against fresh flowers from a plant known to give intermediates only and recorded as grade 1·5. Grade 1 is white. The lobe of the petal is clear ivory white, but the outer edge, where it is exposed in the bud, is a pale greenish yellow. When the petal is very small, practically the whole of it may be exposed in the bud, and a true white may then occasionally be graded as 1·5 or 2.

During the seasons 1926 and 1927 segregating families were classified for corolla colour in the field, and were recorded simply as "yellow," "pale," or "white." During 1928 and 1929 the series of grades shown in Plate X was painted, and thereafter segregating families were classified by collecting five flowers per plant in the early mornings, and matching against the painted standards in the laboratory.

Almost all yellow-flowered types have large petals, and all white-flowered types so far recorded have small petals. Pale-flowered types have large, or moderately large petals.

Petal length was measured for convenience from the point of insertion of the bract to the tip of the longest petal. The mean of five results was taken.

Petal length is a convenient estimate of petal size and ranges from 60 mm. down to 20 mm. In a number of families the maximum width of petal was also measured, and in all cases the correlation between length and maximum width was of the order of $+ 0·9$.

Time could not be spared to obtain petal measurements from progeny rows of all parental types, and in such cases the petal length of the parent

[1] "Pale" means Leake's "pale" unless otherwise stated.

plant employed is given. Where information from progeny rows is available, the range in petal length is given. Parental frequency arrays are tabulated with the hybrid progeny.

Lint lengths are means of ten maximum halo lengths obtained by combing out the lint on one side of each of ten good plump seeds.

Lint index is the weight of lint in milligrams on 100 seeds. Seed weight is the weight of 100 seeds. Lint per cent. is the weight of lint in grams on 100 gm. of seed. Since seed weight is highly correlated with seed-coat area, lint per cent. is taken as a fair index of the density of lint on the seed.

MATERIAL.

The following table includes the types used as parents, and the extracted types used to complete the series of standard corolla-colour grades.

Type	Corolla grade	Petal length	Species and variety	Symbol used
Million Dollar ...	9	48–53	G. Nanking	M.D.
Burma Laciniated ...	9	42	G. arboreum near var. neglecta	B.L.
Burma Khaki ...	9	—	G. Nanking	B.K.
Cawnpore Yellow ...	8	44	G. arboreum var. neglecta	C.Y.
Abu Hareira ...	8	36	G. Nanking var. soudanensis	A.H.
1027	8	40	G. obtusifolium var. Wightiana	1027
An F_2 segregate from C.W. × N 289 ...	7	42	—	9–47
Ditto	6	37	—	9–39
N 289	5–6	24–33	G. herbaceum	N 289
An F_2 segregate from C.W. × N 289 ...	5	34	—	14–104
Ditto	4	32	—	8–12
An F_2 segregate from M.D. × G.C. ...	3	53	—	740
Cernuum	2	48–57	G. arboreum var. assamica	G.C.
Burma Spotless ...	2	38–41	G. Nanking	B.S.
An F_1 of B.S. × C.W.	1·5	41	—	236
Cawnpore White ...	1	27–39	G. arboreum var. rosea	C.W.
1304	1	24	G. Nanking	1304

The specific names are those of Watt (1907).

THE EXPERIMENTS.

I. COROLLA COLOUR MULTIPLE ALLELOMORPH SERIES.

Yellow × Yellow.

1. Million Dollar × Cawnpore Yellow (9 × 8).
   Gave yellows only, in F_1, F_2 and F_3 (not graded).

2. Abu Hareira × N 289 (8 × 5–6).
   F_1 was intermediate with 27 plants grade 6, and 6 plants grade 7.

In F_2 78 plants were graded, giving

	Grade				Total
	5	6	7	8	
Frequency	3	31	36	8	78

Yellow × Pale.

1. Million Dollar × Cernuum (9 × 2).

A single F_1 plant was yellow flowered, grade 7.

F_2 and F_3 were classified into pale and yellow, giving in F_2

62 yellows : 23 pales

graded as follows:

	Grade								
	1	2	3	4	5	6	7	8	9
Frequency	—	19	4	—	—	—	16	38	8

F_3's were classified as "yellow" and "pale" without grading. Nine F_2 plants were tested, of which 4 pales bred true; while of five yellows, two bred true and three gave 24 yellows : 7 pales.

The F_3 families were examined carefully to see whether any white-flowered segregates appeared. None was observed.

2. Burma Khaki × Cernuum (9 × 2).

This cross behaved in the same manner as the previous one.

F_1: yellow (not graded).

F_2:

Family	Yellow	Pale	Total
728	19	5	24
732	15	4	19

In F_3 four families from plants with yellow corollas gave yellow only. Ten families from plants with yellow corollas gave

153 yellows : 32 pales

Expected 139 : 46

The number of recessives is significantly less than the number expected on a 3 : 1 basis. Three families were grown from F_2 plants having pale yellow corollas. Of these two bred true, while one gave 22 pales and 5 yellows where no yellow-flowered plants were expected. This may have been due to a certain amount of natural crossing. If the results were due to the action of more than one factor, more than one type of segregation would be expected among the heterozygotes, but the F_3 families from F_2 yellows gave no evidence of this.

3. Cawnpore Yellow × Cernuum (8 × 2).

F_1: yellow (not graded).

F_2 gave 187 yellows : 60 pales, almost exactly 3 : 1

A back-cross to Cernuum gave

$$19 \text{ yellows} : 20 \text{ pales}.$$

4. N 289 $\times$ Burma Spotless (6×2).

F_1: yellow (grade 5).

A small F_2 family was grown, and also back-crosses to N 289, and to a white-flowered segregate from an F_3 family of Burma Spotless $\times$ Cawnpore White, known as 1304. Flowers were picked and graded against the colour scale shown in Plate X. The results are summarised in Table I.

Back-crossing the F_1 to the yellow parent gave yellows only. Back-crossing to white gave a close approach to a $1:1$ ratio. The F_2 gave a bimodal curve with no pales lower than grade 2. The plant classified as grade 3 gave 2 flowers graded 4, and 3 flowers graded 3. It is therefore classed as yellow, giving in all:

$$19 \text{ yellows} : 7 \text{ pales}$$
$$\text{Expected} \quad 19 \cdot 5 \quad : 6 \cdot 5$$

This is the nearest approach in these experiments to the overlapping distributions for corolla colour so common in New World cottons (Harland, *loc. cit.*).

The back-cross to 1304 (yellow $\times$ pale) $\times$ white forms a critical test of the hypothesis that yellow, pale, and white form a multiple allelomorph series. The back-cross gave yellows and pales only, and the pales were similar to heterozygous pales from pale $\times$ white F_1's and F_2's.

Yellow $\times$ White.

1. Million Dollar $\times$ Cawnpore White (9×1).

F_1: yellow (not graded).

Eleven F_2 families gave

$$1416 \text{ yellows} : 472 \text{ whites}$$

exactly $3:1$. Four back-cross families gave

$$226 \text{ yellows} : 242 \text{ whites}$$
$$\text{Expected} \quad 234 \quad : 234$$

2. Cawnpore Yellow $\times$ Cawnpore White (8×1).

F_1: yellow (not graded).

Thirteen F_2 families gave

$$840 \text{ yellows} : 268 \text{ whites}$$
$$\text{Expected} \quad 831 \quad : 277$$

A back-cross to the whites gave

$$120 \text{ yellows} : 112 \text{ whites}$$
$$\text{Expected} \quad 116 \quad : 116$$

Thirty-four F_3 families were grown from selfed seed. Of those grown from yellow-flowered parents, 12 gave yellows only, and 17 segregated into yellow and white, giving

$$571 \text{ yellows} : 178 \text{ whites}$$

Expected   561·75   : 187·25

Five families were grown from white-flowered plants and all bred true to white, save for 3 yellow-flowered plants in one family, no doubt vicinists.

3. N 289 × Cawnpore White (6 × 1).

F_1: yellow, grades 5 and 6.

F_2 families and back-crosses to N 289 and to Cawnpore White were grown and flowers were graded against the colour scale (Plate X).

The results are shown in Table II.

The back-cross to the yellow parent gave all yellows.

In the F_2 and in the back-cross to white a few pales (grades 1·5 and 2) were recorded. These were all from plants which gave flowers with very small petals. Consequently the strip of petal exposed in the bud formed a much larger proportion of the whole than in normal-sized petals, and the pale greenish yellow on the margin appeared to be diffused almost throughout the petal. From all these plants some flowers were obtained which were definitely grade 1, whereas true pales never give grade 1 flowers. Plants recorded as 1·5 and 2 in these families were all examined at intervals during the season, and it was concluded that they were true whites.

Two batches of seed were sown for the F_2. The first batch was old seed, and only about 25 per cent. germinated. A second batch of fresh seed was then sown in order to get a reasonably large F_2.

Among the plants grown from old seed there were 82 yellows, and 42 whites, a significant excess of whites. Among the plants grown from fresh seed there were 87 yellows and 29 whites, exactly 3 yellows : 1 white.

In the back-cross to white, where the F_1 was used as pollen parent, there were 258 yellows : 315 whites, again a significant excess of whites. The reciprocal back-cross gave 548 yellows : 561 whites, approximately equal numbers.

Apparently the **y** gene is correlated with longer viability of the sporophyte, and also with a greater rate of pollen tube growth of the gametophyte.

4. 1027 × Cawnpore White (8 × 1).

F_1 grade 6.

An F_2 and a back-cross to each parent were grown. Clear segregation

TABLE I.

Corolla colour in F_2 and back-crosses to N 289 and 1304 of Burma Spotless × N 289.

Type	Grade 1·5	2	3	4	5	6	7	Total	Yellow	Pale	χ^2	P
F_1 × N 289	·	·	·	59	102	29	1	191	191	·	0·11	·7
F_1 × 1304	14	28	·	2	6	16	15	81	39	42	0·08	·8
F_2	·	7	1	4	10	3	1	26	19	7		

TABLE II.

Corolla colour in F_2 and back-crosses of N 289 × Cawnpore White.

Type	Grade 1	1·5	2	3	4	5	6	7	8	Total	Yellow	White	χ^2	P
F_1 × N 289	·	·	·	5	48	72	27	2	·	154	154	·	5·67	0·02
F_1♂ × Cawnpore White ♀	315	3	·	2	85	128	43	·	·	573	258	315	0·15	0·7
F_1♀ × Cawnpore White ♂	558	1	·	3	161	253	125	6	1	1109	548	561	0	—
F_2 (good germination)	28	·	·	·	19	27	25	12	·	116	87	29		
F_2 (bad germination)	35	6	1	8	23	20	19	12	1	124	82	42	5·2	0·02

TABLE III.

Corolla colour in F_2 and back-crosses of 1027 × Cawnpore White.

Type	Grade 1	2	3	4	5	6	7	8	9	Not graded Yellow	Not graded White	Total	Yellow	White	χ^2	P
F_1 × 1027	·	·	·	1	4	4	44	20	5	·	·	78	78	·	0·44	0·5
F_1 × Cawnpore White	10	·	·	·	2	2	7	·	·	9	6	36	20	16	Very small	·
F_2	26	·	·	·	2	14	57	13	·	41	17	170	127	43		

into yellow and white occurred. Flowers from about 200 plants were graded in the laboratory (see Table III).

A back-cross to 1027 gave all yellows.

A back-cross to Cawnpore White gave

| | 20 yellows | : 16 whites |
| Expected | 18 | : 18 |

The F_2 gave

| | 127 yellows | : 43 whites |
| Expected | 127·5 | : 42·5 |

Pale × Pale.

A small family of Burma Spotless × Cernuum (2 × 2) gave nothing but pale-flowered plants in F_2 (not graded).

Pale × White.

1. Cernuum × Cawnpore White (2 × 1).

   Four F_1 plants were grade 2.

   The F_2 gave pales and whites only (see Table IV).

   Whites were quite easily distinguishable from the rest when examined in the laboratory. In a family of 53 plants there were 45 pales ranging from grade 1·5 to grade 3, and 8 whites, grade 1. The whites made up less than one-quarter of the whole, but the deficiency is not significant.

TABLE IV.

Corolla colour in F_2's of crosses of pale and white.

Type	Grade				Pale	White	Total	χ^2	P
	1	1·5	2	3					
G.C. × C.W.	8	17	27	1	45	8	53	2·77	0·10
B.S. × C.W.	6	7	7	.	14	6	20	0·27	0·6
Total	14	24	34	1	59	14	73	1·32	0·25

2. Burma Spotless × Cawnpore White (2 × 1).

   F_1 was grade 1·5, and was taken as the standard of this grade.

   A small F_2 was grown, giving

   14 pales (grades 1·5 and 2) : 6 whites

(see Table IV).

Summary.

1. Crosses of yellow × yellow gave yellow only in F_1, F_2 and F_3.

2. Crosses of yellow × pale gave yellow in F_1, and 3 yellow : 1 pale in F_2, and in a back-cross to pale or white gave 1 yellow : 1 pale.

3. Crosses of yellow × white gave yellow in F_1, and 3 yellow : 1 white in F_2, and in a back-cross to white gave 1 yellow : 1 white.

4. A cross of pale × pale gave pale only in F_1 and F_2.

5. Crosses of pale × white gave pale in F_1, and 3 pale : 1 white in F_2. Yellow, pale, and white, therefore, form a multiple allelomorph series.

6. F_1's are usually intermediate between the parents in corolla colour, approaching the deeper-coloured parent.

II. Petal size and corolla colour modifiers.

Long Yellow × Short White.

1. Cawnpore Yellow × Cawnpore White (grade 8, 44 mm. × grade 1, 34 mm.).

F_1 was yellow, and a note was made that it was intermediate in petal length between the parents. No measurements were taken. F_2 results are given in Table V. In F_2 (1927) whites gave a frequency array very similar to that of the parental white, with no long whites. The frequency array of the yellows, on the other hand, overlapped the distribution of the short whites very considerably. The 1926 F_2 showed similar distributions shifted about 6 mm. higher in the scale. Since the F_2's were all grown from a series of sister F_1 plants, and since heterozygous F_3 families grown in 1927 from seed of 1926 F_2 plants behaved like the 1927 F_2, the shift must have been due to environmental effects (see Tables V–VIII). The 1926 F_2 was grown on land which had been out of cultivation for some years. All subsequent plantings were on land that had carried one or more crops. This may account for the difference. This F_2 is the only family grown in 1926 for which petal size data are given. Progeny rows of Cawnpore White grown in 1927, 1928 and 1929 agreed very well, and families of the 1927, 1928 and 1929 plantings are therefore assumed to be fairly comparable.

F_3 progenies of Cawnpore Yellow × Cawnpore White were grown in 1927 from selfed seed of 1926 F_2 plants. Frequency arrays are given for these families in Tables VI–VIII. All homozygous yellows had long petals and gave long-petal progeny only. The petal length of F_2 plants which proved to be homozygous yellows ranged from 42 mm. to 51 mm., with a mean of 48·1 mm. No selfed seed was obtained from the shortest yellows, but families from yellows with a petal length close to the modal petal length of F_2 whites proved to be heterozygotes, and gave long yellows and short whites. No cross-over families were obtained. The petal length of F_2 plants which proved to be heterozygous yellows ranged

TABLE V.

Petal length and corolla colour in F_2 of Cawnpore Yellow × Cawnpore White and in F_2 and back-cross of Million Dollar × Cawnpore White. Parental petal lengths added for comparison.

Type	Corolla	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	Total
Cawnpore Yellow	Y											1								1
Cawnpore White					2	4	24	16	3											49
Million Dollar																1	1		1	3
Cawnpore Yellow × Cawnpore White F_2 1926	Y				7	4	5	9	24	25	15	35	23	19						155
Cawnpore Yellow × Cawnpore White F_2 1927	Y					2	5	3	18	8	5	1	1							43
" " "	y						5	11	18	25	15	11	6	3	1					95
" " "	y			6	4	2	2	2	6	4	5	1								26
Million Dollar × Cawnpore White F_2	Y				5	17	24	46	119	163	194	229	165	144	103	67	31	19	9	1335
Million Dollar × Cawnpore White F_2	y		10	39	67	107	85	57	45	17	6	1	2	2						438
(Million Dollar × Cawnpore White) × Cawnpore White	Y						11	11	27	52	59	42	12	10						224
(Million Dollar × Cawnpore White) × Cawnpore White	y	1	16	40	57	73	39	6												232

Petal length (mm.)

TABLE VI.

Petal length in homozygous yellow F_3 families. Cawnpore Yellow × Cawnpore White.

Family	Parental petal length	34	36	38	40	42	44	46	48	50	52	54	Total
75–42	51					1	2	4	5	2	1	1	16
75–40	51					1	3	5	4	3	1	1	18
75–20	50			1	2	6	13	17	13	8			60
75–51	50						1	3	4	2			10
76–2	50				6	8	14	12	8	3			51
75–31	49			6	8	7	12	14	12	8	4		71
75–9	47			3	5	6	6	13	12	1	3		49
76–23	43				1	6	6	2					15
76–3	42			2	1	6	5	4	2	1	1		22
75–406	?	1	1	2	1	2	2	2					11
76–4	?			4	2	3	2	5	5				21
76–24	?			6	2	3	3	4					18
Total		1	1	24	28	49	69	85	65	28	10	2	362

Petal length (mm.)

TABLE VII.

Petal length and corolla colour in F₃ families segregating for yellow. F_3 *Cawnpore Yellow × Cawnpore White.*

Family	Parental petal length		Petal length (mm.) 22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	Total
75-7	50	Y					1	1			4	5	3			2				15
		y							2											4
76-20	48	Y					1	1		2	2	4	4	7		1				20
		y								1	1									4
76-7	47	Y				1		1	1	1	1	3	3		3					9
		y				1		1	1											3
75-30	46	Y				1		2	2	2	3	6	3	6	3	2	1			29
		y				1	4	5	1											11
75-14	45	Y					1	2	1	3		4		3	1	1				14
		y						2	2											7
75-17	45	Y				2	7	3	2	3	14	14	23	25	26	25	8	8	2	153
		y			1		1	2	2	3	3	1	1	3	1	1			3	43
75-16	44	Y				2	7	3	10	8	8	11	21	37	22	25	14	7		150
		y					1	11		14	3	1								38
76-14	44	Y			1	1	1	5	12	4	1	2		2						10
		y																		3
75-12	43	Y					1	2	1	2	3		1	1		1				9
		y				2	1	1	1											5
75-26	43	Y						1		6	1		1			2	3			8
		y					3	1	5		3		1		1	1				4
75-19	43	Y			1	4	3	1	5	4	6	5	4	4	1	1		1		36
		y				1	4	6	5		4			1	2		3			20
75-33	43	Y					5	3	2								1			20
		y										3								5
75-54	43	Y					2	2	1	2	3	3	1	2	1	2				12
		y						1	1		1	2				1				3
75-11	42	Y		1		3				2	1	5	1	4	1	1				7
		y																		3
76-6	41	Y			1	3	1	2	2	3	2	2	1	2	1			1		19
		y	1		1	1		2		4	4	5	1	2	1	1				6
76-13	?	Y		1	1	2	1		2				2	2	1					16
		y								3	3				3					8
75-378	?	Y			2		2	3	1	4			5	2	3	1		1		14
		y					1		1										5	5
Total		Y	1	2	6	19	3	14	17	37	57	70	71	96	60	65	27	17	5	541
		y					29	42	38	23	10	1	1							172

from 41 mm. to 50 mm., with a mean of 44·5 or nearly 4 mm. shorter than homozygous yellows. The progeny of F_2 whites were all whites with the exception of two plants, no doubt vicinists, which had yellow petals. All had short petals, and gave frequency arrays very similar to that of the parental white. Selfed seed was not obtained from the longest whites, but no long petals were obtained in the progeny of a plant with petals 41 mm. long.

To sum up: there is no evidence that crossing-over occurs between the basic gene for petal length and the corolla colour gene **Y**.

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TABLE VIII.

Petal length in homozygous white F_3 *families.*

Cawnpore Yellow × Cawnpore White.

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Family	Parental petal length	Petal length (mm.)									Total
		24	26	28	30	32	34	36	38	40	
75–461	41	.	.	.	.	1	1	3	.	.	5
75–8	37	.	1	3	2	2	2	.	1	.	11
75–22	37	1	1	4	4	9	8	5	1	.	33
76–5	37	3	6	5	13	5	6	.	1	1	40*
75–422	?	.	.	2	3	5	3	.	1	.	14
Total		4	8	14	22	22	20	8	4	1	103

* Three yellow-flowered plants with 38 mm. petals occurred in this family.

2. Million Dollar × Cawnpore White (grade 9, 52 mm. × grade 1, 34 mm.). F_1 was yellow. No measurements were taken.

An F_2 and a back-cross to Cawnpore White were grown in 1927. Frequency arrays for petal length and corolla colour are given in Table V. In F_2 the dispersion of both yellows and whites is much greater than in the F_2 of Cawnpore Yellow × Cawnpore White grown in the same year. The lower limits are about the same in each case, but the upper limits are much higher. In the back-cross the distribution of whites is similar to the distribution of whites in the 1927 F_2 of Cawnpore Yellow × Cawnpore White. In F_2 two white-flowered plants occur with petals as long as the longest yellow in the back-cross.

The distributions are such as would be expected assuming no crossing-over between **Y** and the main gene for petal length, plus the segregation of several petal length modifiers.

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Long Yellow × Long Pale.

</div>

Million Dollar × Cernuum (grade 9, 52 mm. × grade 2, 54 mm.).

F_1 petal grade 8, petal length 51 mm.

In F_2 corolla grade and petal length were measured on 85 plants, and means and standard deviations determined.

Corolla colour	No. of plants	Mean petal length	Standard deviation of mean
Yellow	62	51·8	0·45
Pale	23	50·4	0·67
Difference	—	1·4	0·81

The yellow-flowered plants had longer petals than the pale-flowered plants. The difference as it stands is scarcely significant, but there was in addition a correlation of $r = + 0·35$, for which $P = 0·01$, between corolla grade and petal length among the yellow-flowered plants[1].

A correlation table of petal length and corolla grade is given in Table IX.

TABLE IX.

Petal length and corolla colour in F_2 *of Million Dollar × Cernuum.*

Corolla grade	Petal length (mm.)									
	42	44	46	48	50	52	54	56	58	60
9	.	.	.	1	.	2	2	3	.	.
8	.	1	.	4	9	5	7	9	2	1
7	1	1	.	3	3	6	2	.	.	.
6	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	1	12	.	.	1
2	.	.	2	6	5	3	2	1	.	.

Long Pale × Short White.

1. Cernuum × Cawnpore White (grade 2, 48–57 mm. × grade 1, 27–39 mm.).

Four F_1 plants gave corolla grade 2, and ranged from 39 mm. to 42 mm. in petal length.

In F_2 53 plants were examined for corolla grade and petal length (see Table X).

The mean petal length of the pales was 6·9 mm. greater than that of the whites. There was a correlation of $r = + 0·31$ $(P = 0·03)$ between corolla grade and petal length among the pales.

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[1] Standard deviations of correlation coefficients are not given, since the correlations are estimated from relatively small samples. The value of P (the probability of obtaining a correlation as large or larger by random sampling from an uncorrelated population) is given from the table published by Fisher (R. A. Fisher, 1928, Table V A).

Correlation coefficients were in all cases calculated from the original data, and not from the grouped data given in the tables.

2. Burma Spotless × Cawnpore White (grade 2, 38–41 mm. × grade 1, 27–39 mm.).

F_1 gave corolla grade 1·5, and had a petal length of 41 mm. Only 20 plants were obtained in the F_2. Petal length and corolla grade data are given in Table XI. Pale-flowered plants again had considerably longer petals than white-flowered plants.

The correlation between corolla grade and petal length among the pale-flowered plants was $r = +0.73$ (P very small).

These five crosses may be considered together.

TABLE X.

Petal length and corolla colour in F_2 *of Cernuum × Cawnpore White.*

Corolla grade	Petal length (mm.)										
	34	36	38	40	42	44	46	48	50	52	54
3	.	.	.	.	.	.	.	.	1	.	.
2	.	.	.	3	4	5	4	5	4	1	1
1·5	.	.	1	1	2	5	7	.	.	1	.
1	.	4	1	.	3	.	.	.	.	.	.

Corolla colour	No. of plants	Mean petal length	Standard deviation of mean
Pale	45	45·0	0·55
White	8	38·1	1·06

TABLE XI.

Petal length and corolla colour in F_2 *of Burma Spotless × Cawnpore White.*

Corolla grade	Petal length (mm.)								
	30	32	34	36	38	40	42	44	46
2	.	.	.	.	.	4	.	2	1
1·5	.	.	1	2	1	3	.	.	.
1	1	2	1	2	.	.	.	.	.

Corolla colour	No. of plants	Mean petal length	Standard deviation of mean
Pale	14	39·2	0·91
White	6	33·0	1·06

Million Dollar and Cernuum are the two types with the longest petals used in these crosses, and may be supposed to carry several plus modifiers of petal length. Million Dollar has a grade 9 corolla, and in Million Dollar × Cernuum F_2 the distribution of corolla grade among the yellows is consistent with the view that there is a little or no segregation of modifiers. There is a significant positive correlation between petal length and corolla grade among the yellows, and pales are shorter than yellows. This is taken to show that the genotype **YY** is responsible for a longer petal than **YYp**, and **YpYp**. Cawnpore White was extracted from the same stock as Cawnpore Yellow and may be supposed to differ from it in

very little besides the corolla colour factor. Cawnpore Yellow has a petal length about 44 mm. and a grade 8 corolla, and may be supposed to differ from Million Dollar in modifying factors responsible for a difference of a grade in corolla colour, and about 5 mm. in petal length.

Burma Spotless has a petal length of about 40 mm. and a pale corolla, and probably has a petal length constitution similar to Cawnpore Yellow.

Million Dollar × Cawnpore White and Cernuum × Cawnpore White will then be expected to give in F_2 some whites longer than the parental whites or those obtained from Cawnpore Yellow × Cawnpore White, and the mean petal length of extracted yellows should be greatest in Million Dollar × Cernuum, intermediate in Million Dollar × Cawnpore White and least in Cawnpore Yellow × Cawnpore White. Means and standard deviations for petal length in these five crosses are given below.

Cross	Yellow		Pales		Whites	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
M.D. × G.C.	51·8	3·56	50·4	3·21	—	—
G.C. × C.W.	—	—	45·0	3·63	38·1	5·60
M.D. × C.W.	44·0	5·12	—	—	34·4	4·86
C.Y. × C.W. (1927 data)	40·4	3·77	—	—	30·6	3·12
B.S. × C.W.	—	—	39·2	3·29	33·0	2·37

The order of the means was as expected, and in all cases the standard deviations from families segregating for modifying factors were greater than those from families homozygous for modifying factors.

Short Yellow × Long Pale.

N 289 × Burma Spotless (grade 5–6, 24–33 mm. × grade 2, 38–41 mm.).

This cross was expected to show repulsion between **Y** and petal length.

F_1 was yellow, grade 5, and two plants had petal length of 35 and 40 mm. respectively.

A small F_2 was grown; also back-crosses to N 289 and 1304, a short petal white extracted from the F_3 of a cross between Burma Spotless × Cawnpore White. Correlation tables for petal length and corolla grade are given in Tables XII–XV; a correlation table is given of petal length and corolla grade in the parents and F_1.

Few of the yellow-flowered plants in either F_2 or back-crosses have petals as short as the yellow-flowered parent, and most of the pales have much shorter petals than the pale-flowered parent. Pales are shorter than yellows, and in F_2 and both back-crosses there is a positive correlation between petal length and corolla grade among the yellows.

Family	Class	Mean	Standard deviation	No. of plants	Petal length-corolla grade correlation	
					r	P
F_2	Yellow	38·6	4·7	19	+0·32	0·15
	Pale	33·0	5·5	7		
$(F_1) \times 1304$	Yellow	39·1	5·0	39	+0·30	0·05
	Pale	31·3	5·1	42		
$(F_1) \times$ N 289	Yellow	34·1	4·2	191	+0·53	Very small

TABLE XII.

Petal length and corolla colour in N 289, Burma Spotless, and the F_1 between them.

Corolla grade	Petal length (mm.)									
	24	26	28	30	32	34	36	38	40	42
				N 289						
6	.	1	.	3	4	.	.	.	.	.
5	.	1	2	1	1	.	F_1	F_1	F_1	.
4	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	Burma Spotless		.
2	.	.	.	.	.	.	.	2	4	1
1·5	.	.	.	.	.	.	.	.	.	.
1	.	.	.	.	.	.	.	.	.	.

TABLE XIII.

Petal length and corolla colour in F_2 of N 289 × Burma Spotless.

Corolla grade	Petal length (mm.)											
	26	28	30	32	34	36	38	40	42	44	46	48
7	.	.	.	.	.	.	.	.	1	.	.	.
6	.	.	.	.	.	1	.	1	.	.	.	1
5	.	.	.	.	3	.	3	.	3	.	1	.
4	.	.	.	.	1	1	1	.	.	.	.	1
3	.	.	.	1	.	.	.	.	.	.	.	.
2	1	1	1	1	1	.	.	1	1	.	.	.

TABLE XIV.

Petal length and corolla colour in a back-cross of (N 289 × Burma Spotless) × 1304.

Corolla grade	Petal length (mm.)												
	20	22	24	26	28	30	32	34	36	38	40	42	44
7	.	.	.	.	.	.	4	3	4	2	1	1	.
6	.	.	.	2	.	.	2	2	.	1	5	2	2
5	.	.	.	.	2	.	1	.	1	1	1	.	.
4	.	.	1	.	.	.	.	.	1	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.
2	1	2	1	2	4	2	4	3	4	2	2	1	.
1·5	.	.	.	2	2	1	4	1	3	1	.	.	.

TABLE XV.

Petal length and corolla grade in a back-cross of (N 289 × Burma Spotless) × N 289.

Corolla grade	Petal length (mm.)										
	24	26	28	30	32	34	36	38	40	42	44
7	.	.	.	.	.	.	.	.	.	.	1
6	.	.	.	.	3	2	5	4	7	4	4
5	.	2	3	12	16	25	15	14	8	6	1
4	2	3	6	12	9	13	11	3	.	.	.

Short Yellow × Short White.

N 289 × Cawnpore White (grade 5–6, 24–33 mm. × grade 1, 27–39 mm.).
In F_1 three plants gave:

Corolla grade	Petal length
5	37
5	35
6	40

In Part I of this paper it was shown that where germination was bad, an F_2 family contained a large excess of white-flowered plants. Since nothing is known of the relative viability of the genotypes **YY** and **Yy**, only the F_2 from fresh seed with good germinating power is considered here.

Correlation tables for F_2 and back-crosses to both parents and to Burma Laciniated, a long yellow with corolla grade 9 and petal length 42 mm., are given in Tables XVI–XIX.

TABLE XVI.

Petal length and corolla grade in F_2 of N 289 × Cawnpore White
(good germination only).

Corolla grade	20	22	24	26	28	30	32	34	36	38	40	42	44	46
8	.	.	.	.	.	.	.	.	1	.	.	.	.	.
7	.	.	.	.	.	.	1	2	1	1	2	3	.	1
6	.	.	1	.	.	1	1	5	6	7	3	1	1	.
5	.	.	.	1	4	5	3	8	2	1	2	.	.	1
4	1	1	1	1	3	2	4	1	1	3	1	.	.	.
3	.	1	.	.	.	.	.	1	.	1	.	.	.	.
2	.	.	1	.	.	.	.	.	.	.	.	.	.	.
1·5	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1	3	4	4	5	2	5	3	1	1	.	.	.	.	.

Means, standard deviations, and petal length-corolla grade correlations for yellows, and means and standard deviations for whites are given below for the F_2 and the three back-crosses.

		Yellows					Whites		
Cross	Mean	Standard deviation	No. of plants	\(r\)	Petal length-corolla grade correlation \(P\)		Mean	Standard deviation	No. of plants
F_2	33·7	5·24	88	+0·48	Very small		26·0	3·25	30
(F_1) × C.W.	33·8	5·13	806	+0·33	Very small		28·9	3·27	876
(F_1) × N 289	35·7	4·28	143	+0·60	Very small				
(F_1) × B.L.	35·9	4·18	221	+0·55	Very small				

Yellows again have longer petals than whites.

In all cases there is a marked positive correlation between petal length and corolla grade among yellows.

TABLE XVII.

Petal length and corolla grade in (N 289 × *Cawnpore White*) × *Cawnpore White*.

Corolla grade	Petal length (mm.)														
	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50
7	.	.	.	.	.	.	.	.	2	1	2	1	.	.	.
6	.	.	.	2	6	13	20	27	26	37	19	11	6	1	.
5	.	.	4	11	28	36	58	67	64	55	35	16	4	2	1
4	.	.	2	6	31	53	53	45	23	20	10	2	.	1	.
3	.	.	.	1	1	1	1	1	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1·5	.	1	.	1	1	.	.	.	.	.	.	.	.	.	.
1	11	63	142	192	191	148	82	38	5	1	.	.	.	.	.

TABLE XVIII.

Petal length and corolla grade in (N 289 × *Cawnpore White*) × N 289.

Corolla grade	Petal length (mm.)												
	22	24	26	28	30	32	34	36	38	40	42	44	46
7	.	.	.	.	.	.	.	.	.	.	1	.	1
6	.	.	.	.	.	1	1	5	6	7	3	4	.
5	1	.	1	4	11	10	13	8	6	5	.	2	.
4	1	3	7	2	10	5	11	6	2	1	.	.	.
3	.	.	1	2	2	.	.	.	.	.	.	.	.

TABLE XIX.

Petal length and corolla grade in (N 289 × *Cawnpore White*) × *Burma Laciniated*.

Corolla grade	Petal length (mm.)											
	26	28	30	32	34	36	38	40	42	44	46	48
8	.	.	.	.	.	2	2	2	1	.	3	.
7	.	1	.	.	8	11	10	10	10	8	2	1
6	.	.	10	19	27	27	21	6	6	.	2	.
5	2	3	4	2	5	9	3	.	1	.	.	.
4	.	1	1	.	.	.	.	.	1	.	.	.

Long Yellow × Short Yellow.

Abu Hareira × N 289 (grade 8, 36 mm. × grade 5–6, 29 mm.).

F_1 ranged from 28–38 mm. in petal length with corolla grades 6 and 7.

F_2's were grown from plants having petal lengths of 37 and 32 mm., and corollas grade 6. Correlation tables of petal length and corolla grade in the two F_2 families are given in Tables XX and XXI. There are

TABLE XX.

Petal length and corolla grade in F_2 *of Abu Hareira* × N 289 (F_1 688).

Corolla grade	Petal length (mm.)												
	24	26	28	30	32	34	36	38	40	42	44	46	48	50
8	.	.	.	.	.	2	.	1	1	.	.	.	1	.
7	.	.	2	2	1	6	5	4	1	1	2	1	.	1
6	1	.	.	.	2	6	4	1	1	4	.	.	.	.
5	.	.	.	.	.	1	.	.	.	.	.	.	.	.

correlations of $r = +0.47$ (P very small), and $r = +0.55$ ($P = 0.01$) between petal length and corolla grade.

The two crosses N 289 × Burma Spotless and N 289 × Cawnpore White show that N 289 carries the basic factor for petal length. In N 289 × Burma Spotless pales are shorter than yellows, and in N 289 × Cawnpore White whites are shorter than yellows.

TABLE XXI.

Petal length and corolla grade in F_2 of Abu Hareira × N 289 (F_1 689).

Corolla grade	Petal length (mm.)													
	24	26	28	30	32	34	36	38	40	42	44	46	48	50
8	.	.	.	.	.	.	1	1	.	1	.	.	.	.
7	.	.	.	1	1	1	1	2	3	1	.	.	.	.
6	.	.	.	.	4	2	2	1	1	.	.	.	.	.
5	.	.	.	1	.	1	.	.	.	.	.	.	.	.

N 289 must be a low-grade yellow and a short-petal type because it lacks modifying factors for petal length and corolla grade, and not because it lacks the main factor. That it is the same group of modifying factors which controls both petal length and corolla grade is shown by the facts that in both N 289 × Burma Spotless and N 289 × Cawnpore White yellows occur of a higher grade than the parental yellow, and that in the two back-crosses to short whites and in the F_2's of Abu Hareira × N 289 there are positive correlations between petal length and corolla grade among the yellows. In these three cases, all the yellows in the family are of the same constitution with regard to the main corolla-colour factor, and the association between colour and size must therefore result from the action of modifying factors.

In parental lines all flowers from a plant have usually the same corolla grade. In hybrid progenies flowers on very many plants fluctuate between two adjacent grades. A number of plants which gave flowers of grades 4 and 5 were picked out and frequency arrays of petal length plotted for "fours" and "fives" separately.

The mean petal length of 135 "fours" was 35.67 mm., and the variance of the mean was 0.2178. The mean petal length of 150 "fives" from the same plants was 37.63 mm. and the variance of the mean 0.1783.

$$\text{Difference} = 1.96 \text{ mm.}$$
$$V \text{ (diff.)} = 0.3961$$
$$\text{Standard Deviation (diff.)} = 0.63$$

Flowers with corolla grade 5 are, therefore, longer than flowers with corolla grade 4 on the same plants.

Summary.

1. The existence of a correlation between petal size and petal colour is confirmed, and it is shown that given a similar genetic background, yellow-flowered plants (**YY**) have longer petals than pale-flowered plants (**YpYp**) and pale-flowered plants (**YpYp**) have longer petals than white-flowered plants (**yy**). The heterozygotes are intermediate in both characters, but nearer to the higher grade and longer-petal parent.

2. Modifying factors which affect petal length also affect corolla grade among yellows, and to a less extent among pales. By a suitable arrangement of modifying factors fairly long whites may be obtained, and also short yellows, but the latter are lower in corolla grade than long yellows.

3. Crossing-over does not occur between genes for petal size and corolla colour.

4. Flowers with a higher corolla grade have longer petals than flowers with a lower corolla grade on the same plant.

III. LINT CHARACTERS AND COROLLA COLOUR.

Yellow × White.

1. N 289 × Cawnpore White.

Lint length was determined on 1263 plants of the back-cross (N 289 × Cawnpore White) × Cawnpore White. Lint index, lint per cent. and seed weight were determined on 509 of these. Lint length was also determined on 101 plants of the F_2.

Frequency arrays for the four characters, from parents, F_1's and F_2, and back-crosses are given in Tables XXII–XXV.

Means and standard deviations for F_2 and back-crosses are given below for yellow- and white-flowered plants separately:

Family	Character	Yellow-flowered M	$\sigma (M)$	White-flowered M	$\sigma (M)$	Difference	σ (diff.)
F_2 *ex* C.W. × N 289	Lint length	24·34	0·22	23·96	0·39	+0·38	0·45
(C.W. × N 289) × C.W.	Lint length	24·75	0·063	24·52	0·058	+0·23	0·086
(C.W. × N 289) × C.W.	Lint index	3·57	0·010	3·81	0·014	−0·24	0·048
(C.W. × N 289) × C.W.	Seed weight	6·93	0·080	6·92	0·090	+0·01	0·121
(C.W. × N 289) × C.W.	Lint per cent.	52·34	0·447	56·19	0·461	−3·85	0·643

Yellow-flowered plants have very slightly longer lint than white-flowered plants. In the back-cross the difference of 0·23 mm. in favour of the yellow-flowered plants is nearly three times its standard deviation and must be judged significant. Seed weights are the same on yellow-flowered and white-flowered plants, but both lint index and lint per cent.

TABLE XXII.

Lint length (mm.) of parents, F₁'s, F₂, and back-cross of Cawnpore White × N 289.

Type		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Total	Mean
C.W.		1																		1	..
N 289																1		1	1	3	..
F₁	Y				4	9	14	10	17	11	4	2	2							73	24·34
F₂	Y				2	3	5	5	7	2	2	1	1							28	23·96
(C.W.×N 289)	Y				5	37	91	131	144	121	49	21	5	1	1					606	24·75
×C.W.	y				11	42	115	172	143	106	53	14	1							657	24·52

TABLE XXIII.

Lint index (mm.) of parents, F₁, and back-cross of Cawnpore White × N 289.

Type		2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	Total	Mean
C.W.				1						1	..
N 289							1			1	..
F₁			1		1	1				3	..
(C.W.×N 289)	Y	1	15	49	106	66	22		2	261	3·57
×C.W.	y	2	4	34	80	73	43	7	5	248	3·81

TABLE XXIV.

Seed weight of parents, F₁, and back-cross of Cawnpore White × N 289.

Type		4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	Total	Mean
C.W.							1									1	..
N 289								1								1	..
F₁								1	2							3	..
(C.W.×N 289)	Y		2	6	13	44	53	42	50	30	18	2		1		261	6·93
×C.W.	y	1		10	16	37	46	46	47	22	14	6		2	1	248	6·92

TABLE XXV.

Lint per cent. of parents, F₁, and back-cross of Cawnpore White × N 289.

Type	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74	76	78	80	82	84	86	88	Total	Mean
C.W.	1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	·
N 289	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	·	·	·	·	·	·	·	·	·	·	·	·	·	1	·
F₁	·	·	·	·	·	·	·	1	1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	3	·
(C.W. × N 289) Y	·	·	2	4	1	4	13	13	24	25	25	33	31	25	16	16	11	7	4	2	1	2	·	·	·	·	·	·	·	1	261	52·34
× C.W. y	·	1	1	2	2	2	2	7	16	13	16	22	23	33	30	23	18	11	11	7	2	4	3	2	·	·	·	·	·	·	248	56·19

TABLE XXVI.

Lint length (mm.) in Million Dollar × Cawnpore White, Parents and F₂.

Type	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Total	Mean
Million Dollar	·	·	·	·	·	·	·	·	·	·	·	·	·	1	·	1	·
Cawnpore White	1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	·
F₂ Y	·	7	10	32	27	50	55	35	22	33	8	9	5	1	1	295	27·04
y	·	3	4	5	16	11	10	9	9	3	4	1	·	·	·	75	26·57

are about 6 per cent. greater on white-flowered plants than on yellow-flowered plants. It may be inferred, therefore, either that there are more lint hairs per unit area of seed coat, or that the hair weight is greater on white-flowered plants than on yellow-flowered plants.

Lint index and seed weight are correlated to the extent of $r = + 0 \cdot 48$ (P very small) among yellow-flowered plants, and $r = + 0 \cdot 58$ (P very small) among white-flowered plants.

There are correlations between lint length and lint per cent. of $r = - 0 \cdot 19$ (P very small) among yellow-flowered plants, and $r = - 0 \cdot 16$ ($P = 0 \cdot 01$) among white-flowered plants. Lint length and petal length are correlated to the extent of $r = + 0 \cdot 16$ (P very small) among yellow-flowered plants, and $r = + 0 \cdot 09$ ($P = 0 \cdot 02$) among white-flowered plants. Among yellow-flowered plants there is a correlation of $r = - 0 \cdot 14$ ($P = 0 \cdot 02$) between lint per cent. and petal length, and $r = - 0 \cdot 17$ (P very small) between lint index and petal length. There are no significant correlations between lint per cent. and petal length, and lint index and petal length among the whites, though what association there is, is negative. No correlation exists between seed weight and petal length. There are no correlations between lint characters and corolla grade.

Taking all the evidence together, it is concluded that the slight difference in lint length between yellows and whites is real, and that both the main corolla-colour factor and the minor corolla colour-petal length modifiers increase lint length, and decrease lint density, or hair weight.

2. Million Dollar × Cawnpore White.

Lint length determinations are available from 370 plants of various F_2 families, 295 plants having yellow flowers, and 75 having white flowers.

Frequency arrays are given in Table XXVI. The mean lint lengths of the two groups were:

	M	$\sigma (M)$
Yellow flowered	27·04	0·145
White flowered	26·57	0·269
Difference	0·47	0·305

Again, yellow-flowered plants have longer lint than white-flowered plants, but the difference is only 1·5 times its standard error, and cannot by itself be judged significant.

DISCUSSION.

The position of Kottur's "pale" will now be considered. The most closely related cross made in the present investigation was 1027 × Cawnpore White (grades 8 × 1). The F_1 was grade 6, and it is suggested that

Kottur's "pale" would match grades 5 and 6 on the present scale. Yellows in F_2 ranged from grades 5 to 8, but there was no clear segregation into two classes. N 289 should be a homozygous "pale" on this scheme. F_1's of N 289 × Burma Spotless and N 289 × Cawnpore White and Abu Hareira × N 289 are grades 5 or 6, and so correspond with expectation on Harland's scheme. The crosses made must have been more complex than Kottur's, for in no case was clear segregation into "pale" and "full" observed.

Kottur's results for petal length show a considerable seasonal effect similar to that observed in the F_2 of Cawnpore Yellow × Cawnpore White, so that direct comparisons between parents and F_2 are of doubtful value. It is unfortunate that the petal length of "pales" is not given separately from that of full yellows. That segregation for a number of petal-size modifiers is taking place is indicated by the fact that the F_2 distributions for yellow and white overlap a great deal more than the distribution of parental plus F_1 yellows overlaps the distribution of parental whites. In the crosses recorded by Leake, on the other hand, there can have been little or no segregation of modifiers since Leake found only two types in F_2, viz. long yellows and short whites.

A general scheme for corolla colour and petal size may be devised which incorporates the main features of Harland's interpretation of Kottur's results:

(1) A multiple allelomorphic series of three members **Y**, **Yp** and **y**, controls the main differences in corolla colour and petal size. **Y** corresponds to Harland's **A** and converts Leake's pale (**Yp**) or white (**y**) to Kottur's pale. **Yp** converts white (**y**) to pale. Minor factors being equal, **Y** converts intermediate petal (**Yp**) or short petal (**y**) to long petal, and **Yp** converts short petal (**y**) to intermediate petal.

(2) A series of minor factors (Harland's **B** and **C** and others) modify both petal length and corolla colour. These factors act in the same direction on both characters giving rise to positive correlations between them.

All the *arboreum* and *Nanking* plants used here carried a number of plus modifiers, which raised the corolla colour to full yellow in the presence of **Y**. 1027, the *obtusifolium* used, was a full yellow, but must have carried a different set of modifiers from those found in the *arboreum* and *Nanking* plants, since Kottur's pale appeared in F_1 and F_2. N 289, the *herbaceum* used, carried fewer plus modifiers than any other type.

When Kottur's pale and Leake's pale are growing side by side in pure culture, there is no difficulty whatever in distinguishing them. In the

F_2 of N 289 × Burma Spotless, the frequency arrays of Kottur's pale and Leake's pale just met at grade 3, but even in this cross, only one doubtful plant occurred, and it is not likely that any arrangement of modifiers could mask the segregation of Kottur's pale (for which the term "light yellow" is suggested) and Leake's pale in the manner so common in families of New World cottons segregating for **Y** and **y**.

Comparison with the results obtained by Harland (*loc. cit.*) from New World cottons is instructive. There are only two allelomorphs of the main factor in New World cottons, yellow (**Y**) and pale cream (**y**). Nothing corresponding to the ivory white of Cawnpore White has been found in New World cottons. There is some evidence (for the use of which I am indebted to Dr Harland) that pale cream petals are slightly shorter than yellow petals. No correlation has been found between corolla grade and petal length within the yellow class. Corolla-colour modifiers, therefore, are without effect on petal length.

In New World cottons the corolla-colour modifier complex was found to be typical of the species. Asiatic cottons seem to fall naturally into two groups:

(1) *G. arboreum* and *G. Nanking*, in which all three allelomorphs of the main factor occur.

(2) *G. obtusifolium* and *G. herbaceum*, in which all recorded varieties have yellow corollas.

Field observations on a considerable range of forms indicate that *arboreum* and *Nanking* yellows are usually deeper in colour and longer in petal than *obtusifolium* and *herbaceum* yellows, and therefore, presumably, carry a distinct modifier complex.

Kearney (1923) records correlations of $r = + 0.214$ between petal length and corolla colour, and $r = + 0.277$ between corolla colour and lint length in F_2 of an Upland × Egyptian cross. Corolla-colour segregation was complex, and it was not possible to distinguish the main yellow-cream segregation. In the light of Harland's unpublished results referred to above, it may be concluded that the petal length-corolla colour correlation results from the action of the main corolla-colour factor.

The only other published data on the relation between corolla colour and lint length in New World cottons are those given by Burd (1926) for lint length and corolla colour in a small F_2 of a cross between Sea Island yellow and Sea Island white. No difference was observed between the means of the yellow and white classes, but the family was too small to give information of any value.

While the evidence for association between lint characters and corolla characters is at present only very meagre, it is in agreement with statements to be found in many of the reports of the Indian Agricultural Departments, and indicates very valuable aids to the plant breeder.

The main corolla-colour factor evidently has a very considerable effect on the plant, since it is known to affect in Asiatic cottons, corolla colour, petal size, lint length, and weight of lint per unit area of seed coat, and in New World cottons, petal size, and lint length.

It may be noted that Rasmusson (1921) reports an association between corolla colour and corolla size in *Godetia*, where flowers and non-yellow margins (**AA**) have larger corollas than flowers with yellow margins (**aa**).

ACKNOWLEDGMENTS.

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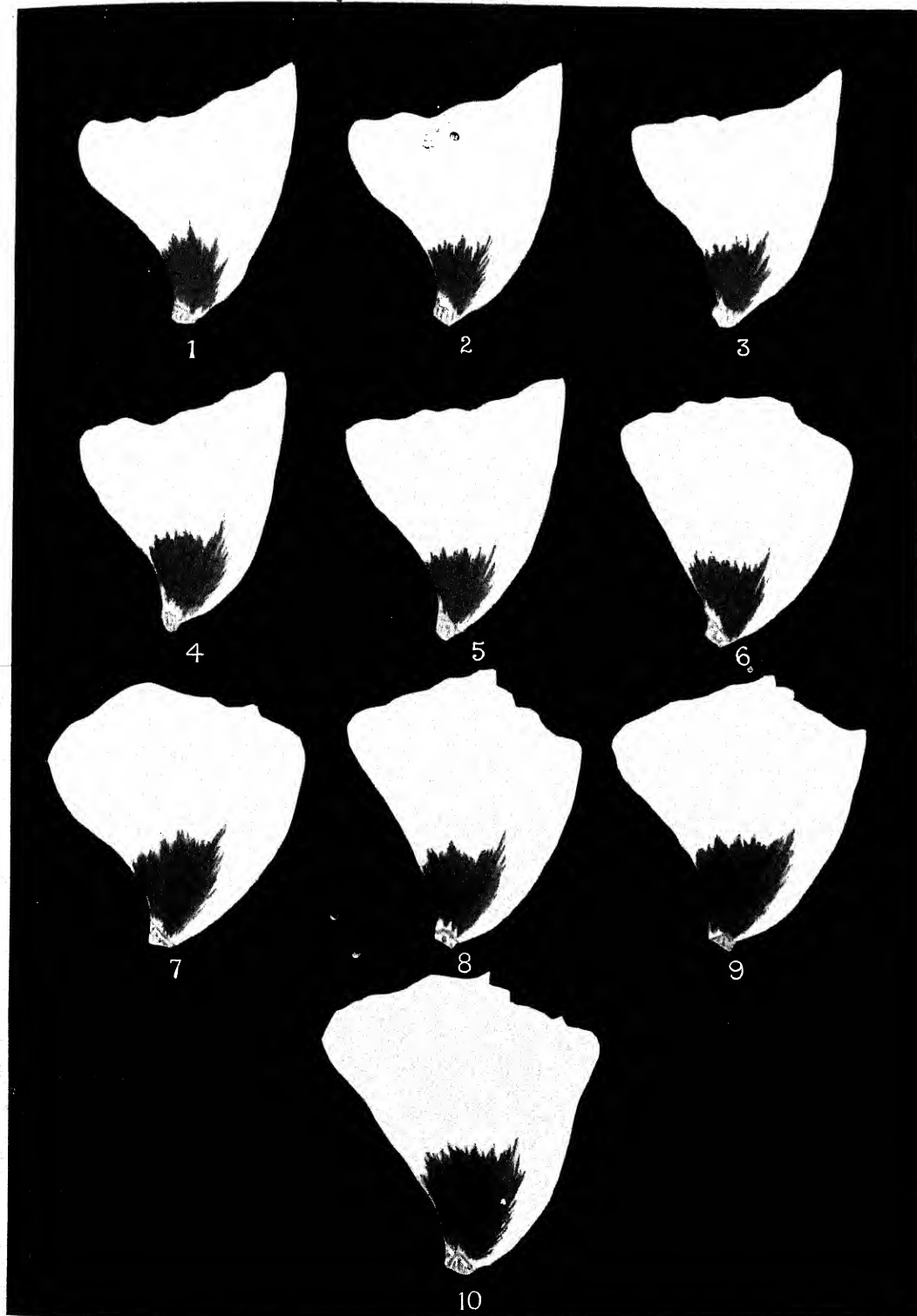
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EXPLANATION OF PLATE X.

Grades of corolla colour in Asiatic cottons.
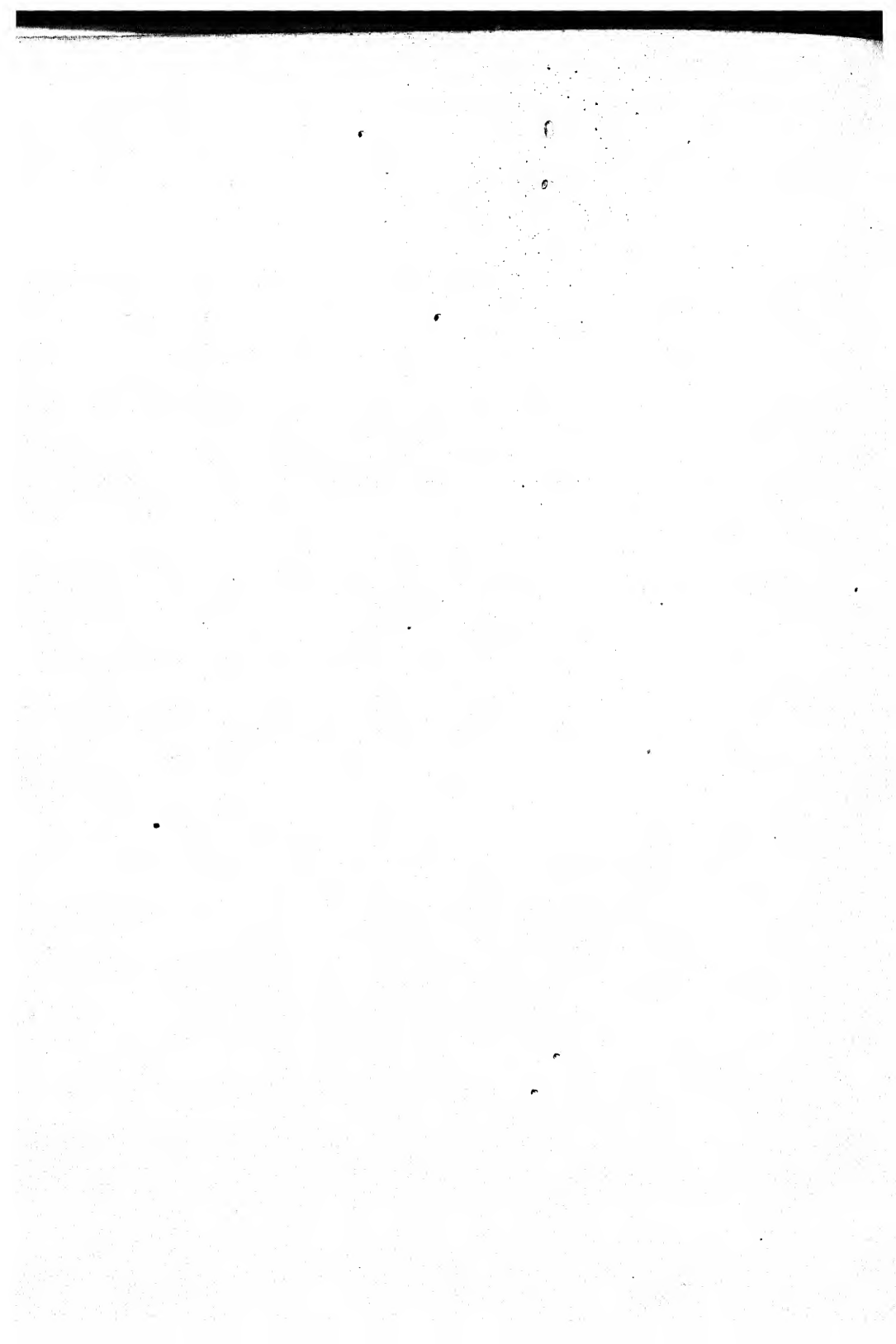
THE INCIDENCE OF STERILITY AMONGST TORTOISESHELL MALE CATS.

By RUTH C. BAMBER (Mrs BISBEE), M.Sc., F.L.S.,
AND E. CATHERINE HERDMAN, M.Sc.

(*University of Liverpool.*)

TORTOISESHELL[1] coat-colour in cats is the heterozygous condition of yellow and black. Tortoiseshell females are fairly common, but in the ordinary course of inheritance tortoiseshell males do not occur. Apparently both black and yellow are sex-linked and so do not normally occur together in the male (Little, 1912 and 1919; Doncaster, 1913; Bamber, 1927 (survey of literature); Bamber and Herdman, 1927 a).

Tortoiseshell males do appear, however, as rare exceptions, and it is generally stated in scientific literature that such males are usually sterile.

Since considerable importance is attached to this sterility in practically all discussions on the origin and gametic constitution of the abnormal colour type, it is of interest to ascertain exactly what data exist on the subject.

The recorded facts are few. Cutler and Doncaster, in 1915, gave a list of the then known adult tortoiseshell males with the available information regarding their fertility. They recorded seven in all. Of these one had died without being mated. Of the remaining six, one was certainly fertile and two were certainly sterile. Another was certainly almost sterile.[2] The remaining two were both said to have sired kittens, but there was no definite proof.

Since this publication, seven other adult tortoiseshell males have come under our notice. One of these, "Lucifer," has been recorded by us elsewhere (Bamber and Herdman, 1927, 1928). The others are not on record in scientific literature, but some of them are registered in the books of the Cat Fancy.

Table I contains all the adult tortoiseshell males hitherto recorded in scientific literature, together with those now recorded for the first time. In order to avoid accidental duplication of records we have given the name of each cat and the name of his owner. We have been able to

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[1] Tortoiseshell here includes tortoiseshell-and-white and tabby-tortoiseshell.

[2] He mated repeatedly without result, but on one occasion a female, after mating with him, produced one kitten. As, however, she had not been kept in confinement after the recorded mating, the paternity of the kitten is uncertain.

TABLE I.

Name of Cat	Name of owner	Remarks on cat	Fertility	Previous records
Samson	Sir Claud Alexander	Mated repeatedly and sired many kittens	Fertile	Doncaster, 1904 and 1913; Cutler and Doncaster, 1915
Unnamed	Sir Claud Alexander	Up to date has mated once only and sired two kittens	Fertile	—
Lucifer	Bamber and Herdman	Mated repeatedly and sired many kittens	Fertile	Bamber and Herdman, 1927, 1928
Bachelor	Sir Claud Alexander	Mated repeatedly with no result. On one occasion one kitten was produced, but since the female had not been kept in confinement the paternity was doubtful	Certainly almost sterile	Cutler and Doncaster, 1915
Benedict	Sir Claud Alexander	Mated repeatedly without result	Sterile	Cutler and Doncaster, 1915
Tom Noddy	Sir Claud Alexander	Mated repeatedly without result	Sterile	—
William	Doncaster	Mated repeatedly without result	Sterile	Doncaster, 1914; Cutler and Doncaster, 1915; Bamber, 1922
Torchlight	Mrs Langdale	Mated repeatedly without result	Sterile	—
King Saul	Mrs Herring	Was believed to have sired kittens, but no definite records	?	Cutler and Doncaster, 1915
Solomon	Sir Claud Alexander	Was reported to have sired kittens, in his youth, but no definite records. As an old cat was too savage to mate	?	Cutler and Doncaster, 1915
Unnamed	Sir Claud Alexander	Savage with females and will not mate	?	—
Tom	Bamber and Herdman	Died unmated at about 10 months old	?	—
Neptune	Sir Claud Alexander	Died unmated	?	Cutler and Doncaster, 1915
Unnamed	Sir Claud Alexander	Died unmated at about 8 months old	?	—

identify the cats recorded by Cutler and Doncaster by reference to Doncaster's original correspondence. For most of the new records we are indebted to Sir Claud Alexander.

Thus, of the fourteen adult tortoiseshell males now on record, three died unmated and one is savage and will not mate. Of the remaining ten, two are reputed to have been fertile but there is no recorded evidence. There are only eight, therefore, of which we have definite[1] knowledge in regard to fertility. Of these, three are certainly fertile, four are certainly sterile and one is certainly almost sterile, probably completely so.

There are no published records of the incidence of sterility among ordinary male cats, but in the course of our own breeding experiments we have used fourteen such males, taken at random from the general population, and all have been fertile.

It does appear, therefore, that the abnormal association of black and yellow in the male cat is correlated with a tendency towards sterility.

Our thanks are due to the Royal Society of London for grants which enable us to investigate the genetics of domestic cats.

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[1] In all these cases matings have been made under definitely controlled conditions, the females having been kept in confinement both before and after mating.
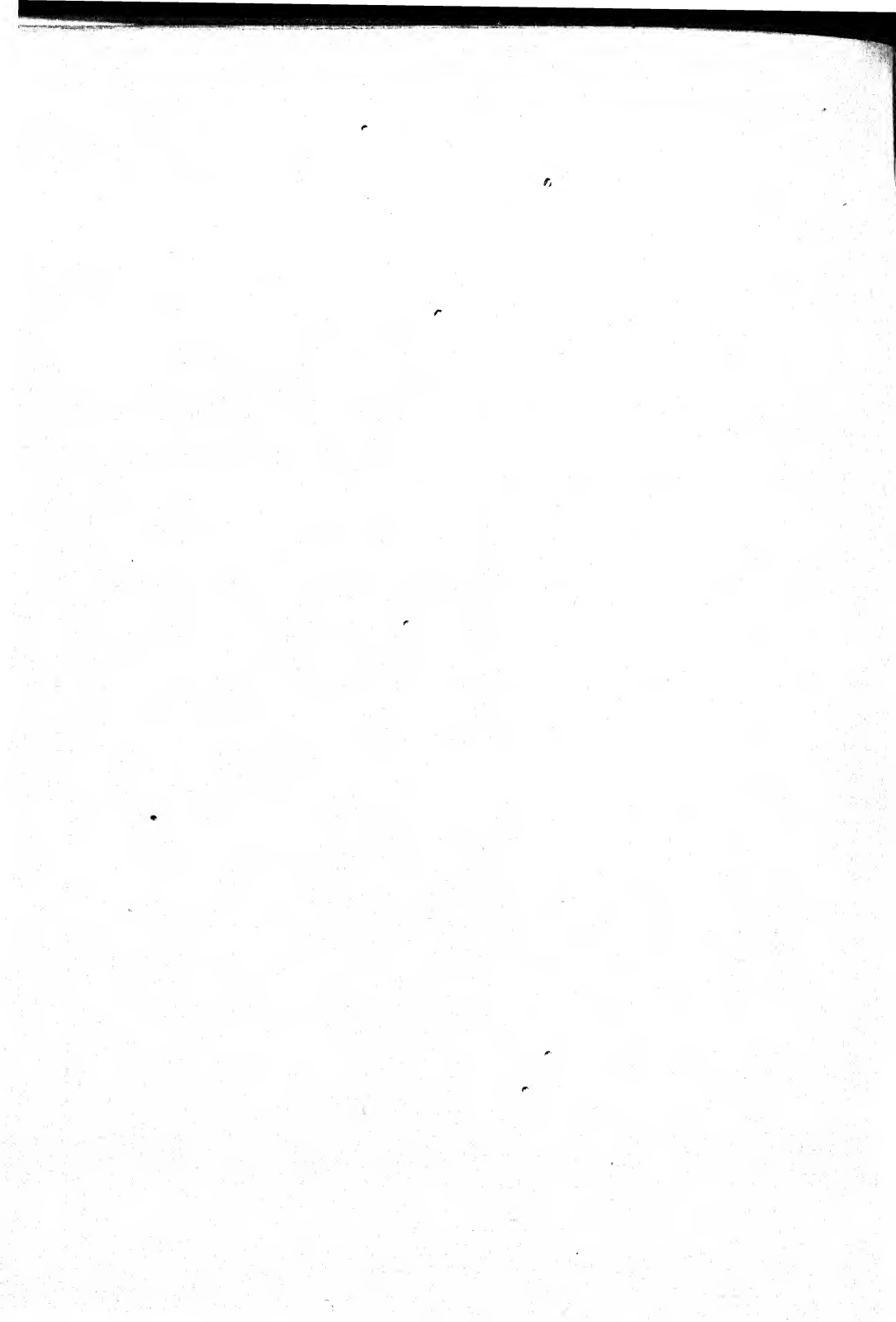
THE CYTOLOGY OF *MATTHIOLA INCANA* R.BR. ESPECIALLY IN RELATION TO THE INHERITANCE OF DOUBLE FLOWERS.

By J. PHILP AND C. L. HUSKINS.

(John Innes Horticultural Institution, Merton.)

(With Plate XI, Sixty-three Text-figures and One Diagram.)

CONTENTS.

I. INTRODUCTION.

THE garden Stock, *Matthiola incana* R.Br., has been in cultivation for very many years, the double-flowered form having been first described by Dodoens in 1568. This form is completely sterile, both ovaries and stamens being metamorphosed into petals, and therefore it can only be obtained from the seed of single-flowered plants.

Miss Saunders has dealt fully with the history and genetics of the Stock in her publications, and it is to her that most of our knowledge of its genetics is due. Since the present study deals with the cytology of *Matthiola* primarily in relation to the inheritance of doubling, a brief account will be given of the salient features of this phenomenon.

Among the single-flowered plants, which are phenotypically similar, strains occur which differ in their breeding behaviour where doubleness is concerned. One race on selfing will produce only singles in its progeny and in all succeeding generations. These pure-breeding singles are generally known as the "pure singles" or "no-d." races. Another strain is heterozygous for doubleness and behaves in a normal Mendelian

manner, giving a proportion of three singles to one double on selfing. The races known as "ever-sporting singles" or "d. races" on selfing produce, according to Miss Saunders, a constant proportion of doubles and singles, about 53–4 per cent. doubles and 46–7 per cent. singles. These singles on selfing will continue to produce the same proportion of doubles and singles in all succeeding generations.

A variety of the d. race called "Snowflake" has given rise to extra-chromosome types which have been studied by Frost (1919, 1927), and by Frost and Mann (1924), and certain of these types give still different ratios of singles to doubles.

It is known that in the d. races, only pollen carrying doubleness effects fertilisation. Snow (1924–5) carried out comparative tests on the pollen germination of the two races, both on artificial culture media and on the stigma; but the results, although they showed that pollen of the no-d. races germinated better than pollen of the d. races, were not con-clusive. Waddington (1929) carried out similar experiments and obtained a mean germination of $44 \cdot 2 \pm 0 \cdot 6$ per cent. for 6857 grains of a no-d. race, and $18 \cdot 65 \pm 0 \cdot 47$ per cent. for 6625 grains of a d. race. These results strongly suggest that there is a factor in the d. race which is lethal to all pollen containing it, thus explaining the non-functioning of pollen carrying singleness.

It was found by Allen (1924) that the diploid chromosome number was 14 in both the no-d. and the d. races, but it was thought that a more extensive study might reveal some cytological explanation of the differ-ence in the genetical behaviour of these two races. Seeds were kindly supplied by Miss Saunders of her no-d. races, d. races and hybrids, and by Dr H. B. Frost of his Snowflake variety and its mutants and hybrids.

The studies of somatic chromosomes have been made entirely by J. Philp, who has also made the observations on the permanent smears of the pollen mother-cells of Miss Saunders' strains. The studies of the pollen mother-cells of H. B. Frost's material, and of their bearing on theories of meiosis, the ovule studies of both lots of material, and the aceto-carmine studies of Miss Saunders' material have been carried out by C. L. Huskins. The genetical hypothesis concerning doubleness has been made by J. Philp. Each author is responsible for the description of his own observations.

II. Material and Methods.

As mentioned, seeds of various varieties and strains were obtained from Miss E. R. Saunders. Of these, three no-d. races, six d. races

including two sulphur-whites, and one hybrid no-d. white × d. cream were examined. Seeds of the ever-sporting variety Snowflake, and of its extra-chromosome mutants and hybrids, were obtained from Dr H. B. Frost. Of these, Snowflake strain No. 1, the mutants "Crenate" No. 2, "Crenatoid" No. 3 a, "Slender" No. 6 a, the hybrids "Crenate" No. 16 a and b, "Smooth" No. 18 a, "Slender" No. 24 c and d, and tetrasomic "Slender" No. 33 have been examined. At diakinesis and later stages of meiosis the chromosomes of all Miss Saunders' strains are of the short and condensed form commonly found throughout the Cruciferae. The chromosomes of the Snowflake variety are long and slender at these stages.

Root tips were taken from the seedlings and fixed in most of the standard fixatives for plant cytology. Benda's fluid gave the best results, and the illustrations, with the exception of one from material fixed in Navashin's fluid, are from Benda material. Ovules could not be fixed satisfactorily in any of the standard fluids tried, but good results were obtained from a new fixative due to L. La Cour, the first of the three he has since published (*Nature*, 1929). Pollen mother-cell smears made according to Taylor's (1924) method were tried, and a number of fixatives, of which La Cour's and modifications of it, again gave the most satisfactory results. Very satisfactory fixations of pollen mother-cells were also obtained by the aceto-carmine method, either temporary as described by Belling, or permanent as described by McClintock (1929), except that prior fixation in acetic alcohol proved unnecessary and undesirable.

III. Observations on somatic chromosomes.

All plants of Miss Saunders' strain were found to have fourteen somatic chromosomes.

On examining a plant of the no-d. race it was found to possess a pair of chromosomes having a sub-terminal attachment constriction and a trabant attached to the shorter arm of each chromosome (Text-fig. 1). These chromosomes are throughout referred to as the "*A*"-chromosomes. Two sister seedlings, the progeny of a d. race, were then examined. One plant showed a similar pair of *A*-chromosomes, each having a trabant (Text-fig. 2). The sister plant, however, possessed a pair of *A*-chromosomes identical with those mentioned in the two previous plants, except that one of the pair had no trabant (Text-fig. 3).

If the following assumptions are made:

(1) that these two chromosomes are carrying factors for singleness (and doubleness), *these factors not being located in the trabant;*

(2) that the pure single plant is homozygous for singleness, **S**;

(3) that the plant with two trabants in the d. race is homozygous for doubleness, **s**;

(4) that the sister plant having only one trabant is heterozygous for singleness, **Ss**, **S** being carried in the chromosome which has lost a trabant;

(5) that pollen containing the chromosome lacking the trabant does not effect fertilisation;

then in the d. race that plant with the two trabants should prove to be a double, and that with the trabant missing should prove to be a single. When the two plants in question flowered, this was in fact found to be the case. A number of double-throwing plants have been examined and in no case have two trabants been seen, whereas in doubles and pure singles both trabants have been observed.

A hybrid resulting from a pure single female crossed with an ever-sporting single shows both A-chromosomes to have a trabant (Text-fig. 4). This is according to expectation on the assumption that in the ever-sporting single only the pollen carrying the A-chromosome with the trabant effects fertilisation. This F_1 plant and all its sister plants ought therefore to have the same chromosome constitution, and on selfing ought to give in the next generation a proportion of three singles to one double. In other words, although one of the A-chromosomes is carrying singleness and the other A-chromosome is carrying doubleness, since both chromosomes have the trabant, pollen containing either of the A-chromosomes will be able to fertilise the ovules, *i.e.* ordinary Mendelian segregation will result. The ever-sporting character is lost.

It would be interesting to know if, on carrying out pollen germination tests, this hybrid pollen would give the same percentage pollen germination as the pure single pollen.

Seed from the F_1 of a cross Trisomic Crenate Single (mutant from Snowflake) × Pure Single was obtained from Dr H. B. Frost. The breeding results of these Crenate singles is in accordance with the view that they are trisomic for the chromosome carrying doubleness. That it is this particular pair of A-chromosomes which is concerned with doubleness was confirmed by the examination of the root tips of a trisomic Crenate single plant raised from this F_1 seed. The plant had three chromosomes of the type in question (Text-fig. 5); the other twelve chromosomes can be paired off with a considerable degree of accuracy into the six types, which correspond very well with those found in the diploids.

Combining Frost's terminology with the cytological observations, D'
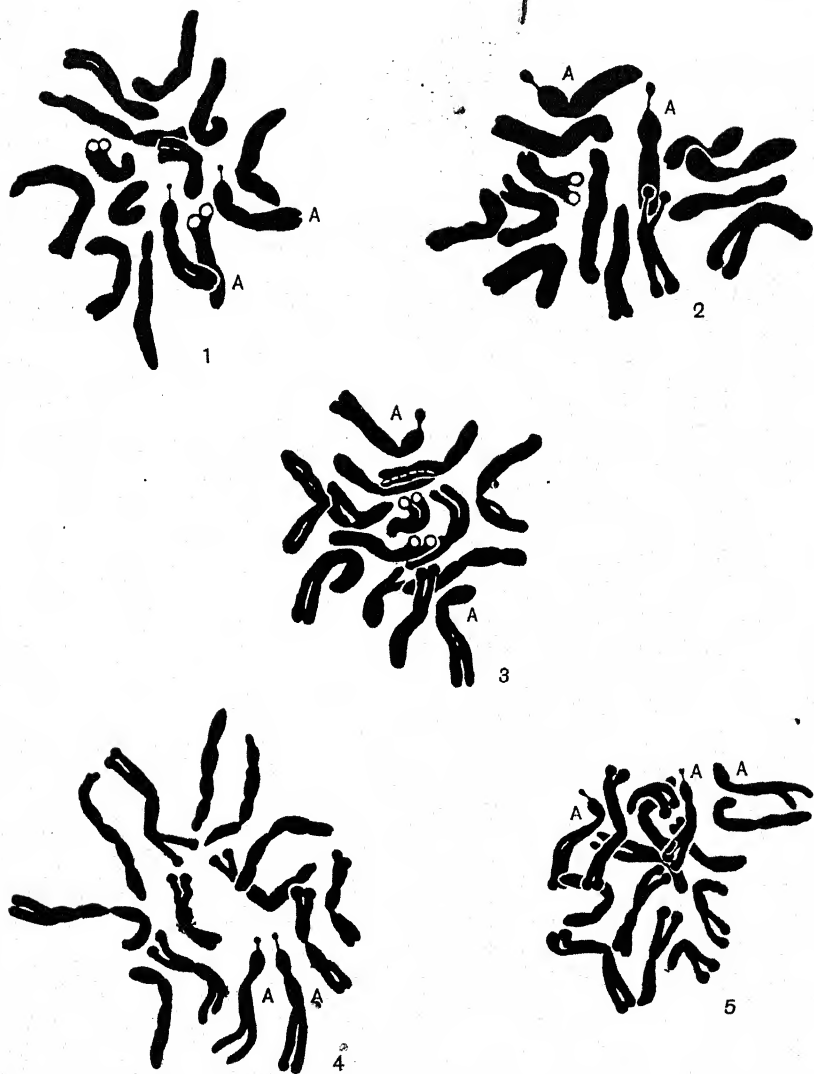
Text-fig. 1. Somatic metaphase from the root tip of a pure single, $2n = 14$. A trabant on each of the two A-chromosomes. ($\times 5500$.)

Text-fig. 2. Somatic metaphase from the root tip of a double, $2n = 14$. A trabant on each of the two A-chromosomes. ($\times 5500$.)

Text-fig. 3. Somatic metaphase from the root tip of an ever-sporting single, $2n = 14$. A trabant on only one of the A-chromosomes. ($\times 5500$.)

Text-fig. 4. Somatic metaphase from the root tip of a hybrid, pure single × ever-sporting single, $2n = 14$. A trabant on each of the two A-chromosomes. ($\times 5500$.)

Text-fig. 5. Somatic metaphase from the root tip of a trisomic crenate single, $2n + 1 = 15$. Note the three A-chromosomes, two of which have a trabant while the third has none. ($\times 5500$.)

represents a chromosome carrying the singleness factor but lacking the trabant, and $D \times d$ are respectively chromosomes carrying singleness and doubleness but both having the trabant.

It may be assumed that the P_1 Crenate single is of the constitution $D'dd$ (using Frost's terminology and combining it with the cytological observations, d being the recessive doubleness, D and D' being the dominant singleness, D' however being singleness carried in the chromosome lacking the trabant), which is most likely, since one would expect the normal chromosome with the trabant to be in duplicate rather than the chromosome which has lost a part. This is supported by the view that pollen carrying the abnormal chromosome does not function, also by the possibility, which will be discussed later, of a selective elimination of ovules containing this chromosome.

The P_1 pure single was DD and the trisomic Crenate single F_1 plant was therefore of the constitution Ddd or $DD'd$ (the pure single parent being DD). Frost considers that it was *probably Ddd* and therefore on the evidence obtained from the diploid plants this F_2 Crenate single plant should have a trabant present on all three of the chromosomes carrying doubleness.

On observation (Text-fig. 5) it was found that two of the chromosomes had a trabant and the third clearly had no trabant. The F_1 plant therefore was more probably $DD'd$.

There is no doubt that, on the evidence obtained from the trisomic, it is these A-chromosomes which are carrying doubleness, and it is believed that in the ever-sporting single one of the A-chromosomes has lost a trabant. The proof of the absence of a trabant is necessarily more difficult than the proof of its presence, and numerous root tips have had to be examined in each of these cases before coming to the conclusion stated above.

A case somewhat analogous to this was found by S. Nawaschin (1927) in *Galtonia candicans,* where one race possessed two chromosomes, each with a trabant, and another race possessed two similar chromosomes but with one of the trabants much smaller than the other. No race was found having two of the smaller trabants, nor was it produced on selfing the heteromorphic race.

Dr M. Navashin also kindly informs us that he has recently found a plant of *Crepis tectorum* in which one member of one pair of chromosomes has lost its trabant and that this plant has about 50 per cent. pollen sterility.

It is therefore not surprising that one of the A-chromosomes in the

Pure Single   Ever-sporting Single   Double   Pure Single ♀ × Ever-sporting Single ♂   Trisomic Crenate Single
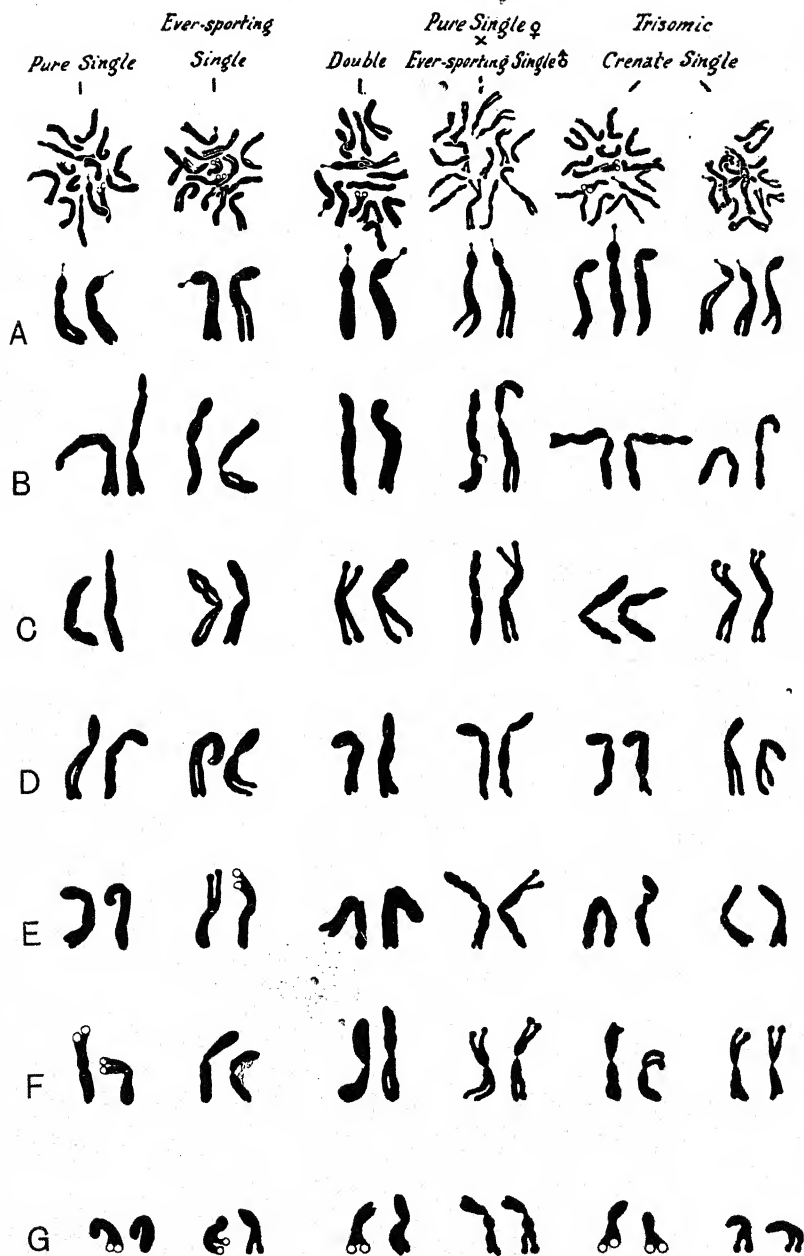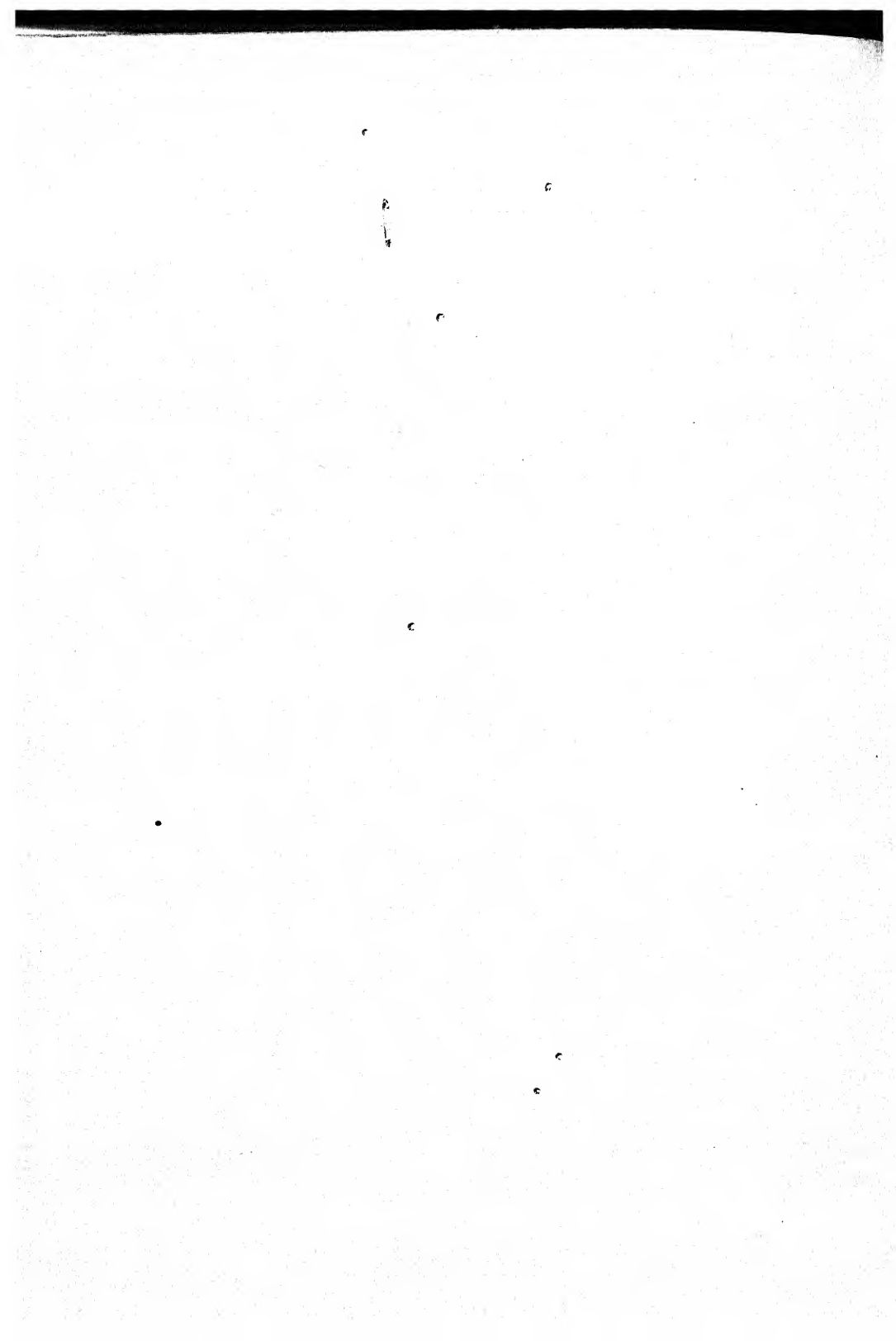
Stock should have lost the trabant, since it is attached to the chromosome by such a fine strand of chromatin, and moreover it has been claimed that the satellited chromosome undergoes fragmentation oftener than others in *Crepis tectorum, C. capillaris* (Navashin, 1926, 1929) and *C. Marschalli* (Babcock and Mann Lesley, 1926).

IV. DESCRIPTION OF SOMATIC CHROMOSOMES.

Plate I shows metaphase plates in cells of the root tips of five races of Stock. The two plates on the right are taken from different root tips of the same plant, which is trisomic for the chromosome carrying the singleness and doubleness factors. This is done in order to prove conclusively that it is the *A*-chromosome which is represented three times, and also to show the difficulty experienced in obtaining a cell in which all the *A*-chromosomes have that end which bears the trabant lying free from the other chromosomes. The plate on the right shows that two of the *A*-chromosomes possess trabants, and the third *A*-chromosome has no trabant. The plate next to it has one *A*-chromosome with the trabant end lying so close to another chromosome that the trabant cannot be seen, although it must be present. For most purposes, however, this plate might be regarded as almost ideal.

Beneath each plate the seven pairs of homologous chromosomes are set out, each type being denoted by a letter. It can be seen that each type is represented in all the races. The *B*-chromosomes are the longest, being about 4μ long, and the *G*-chromosomes are the shortest, being about 2μ long. The most difficult types to distinguish are the *B* and *C*-chromosomes, since they are nearly equal in length and have almost median attachment constrictions.

Chromosome *E* has an almost median attachment constriction and is slightly longer than *F*, which has a median attachment constriction. The shortest type, *G*, has a sub-median attachment constriction, and it is specially noticeable that the longer arm of the chromosome is usually split.

Chromosomes *A* and *D* differ in that *A* is slightly longer than *D* and has the attachment constriction more sub-terminally situated. Although *A* type is readily distinguished from the others, it may be thought that it can be confused with *D* type. A correlation table was made by plotting the length of the short arm against the length of the long arm of the chromosomes, and it was found that *A* and *D* types occupied separate and distinct areas on the table.

These divisions were observed in cells which were located in the same

region of the root tip, and are therefore comparable in that respect. The chromosomes of the double plant where Navashin's fixing fluid was employed are much swollen in comparison with the others where Benda's fixative was used. It would seem at first sight that the chromosomes of the hybrid and of the trisomic Crenate single were longer and thinner than those of the other races, but since there is a distinct difference in the length of the chromosomes in the two cells from the same trisomic Crenate single plant, this must be regarded as being due to differences in fixation. There is therefore no evidence to show that the races having long meiotic chromosomes have longer somatic chromosomes than those of the races with the short meiotic chromosomes. This is important in relation to the theory of the origin of long meiotic chromosomes (Section VIII). Finally, the differences in the size of the trabants in the different cells are considered as probably being due to differences in fixation.

V. Observations on Ovules.

No attempt has been made to study in detail the stages of the prophase in this relatively unfavourable material, but all prophase observations made are in agreement with the parasynaptic interpretation. Amphitene stages, in which most parts of the chromatin threads were associated laterally in pairs, while a few were single, were very clearly observed in some material. At diakinesis seven pairs of chromosomes were found in six different strains of Miss Saunders' material. The chromosomes were, as a rule, closely associated, either at one or both ends, but in some cases one or two pairs had their members very widely separated (see especially Text-figs. 10 and 12). This variation occurs, however, in all the strains examined, and cannot be related to the inheritance of doubling.

In one embryo-sac mother-cell of a no-d. white plant (Text-fig. 7), a fragmented chromosome was observed.

In embryo-sac mother-cells of Crenate singles of Frost's strain, which are trisomic for the doubleness-singleness factors, the 15 chromosomes were found associated at diakinesis either as $7_{II} + 1_I$ or $6_{II} + 1_{III}$. In one cell (Text-fig. 14) the univalent chromosome and one member of one of the bivalents each have a sharply constricted terminal portion. It is tempting to regard these constricted parts as equivalent to the trabants observed in the somatic chromosomes, especially as this plant might be expected to bear trabants on two chromosomes of its trisomic group. Such a conclusion would, however, be very hazardous in view of

the fact that much more favourable material with clearly defined tra-
bants on its somatic chromosomes, such as *Tradescantia* (Darlington,
1929 *c*) and *Tulipa* (Newton and Darlington, 1929), does not show them
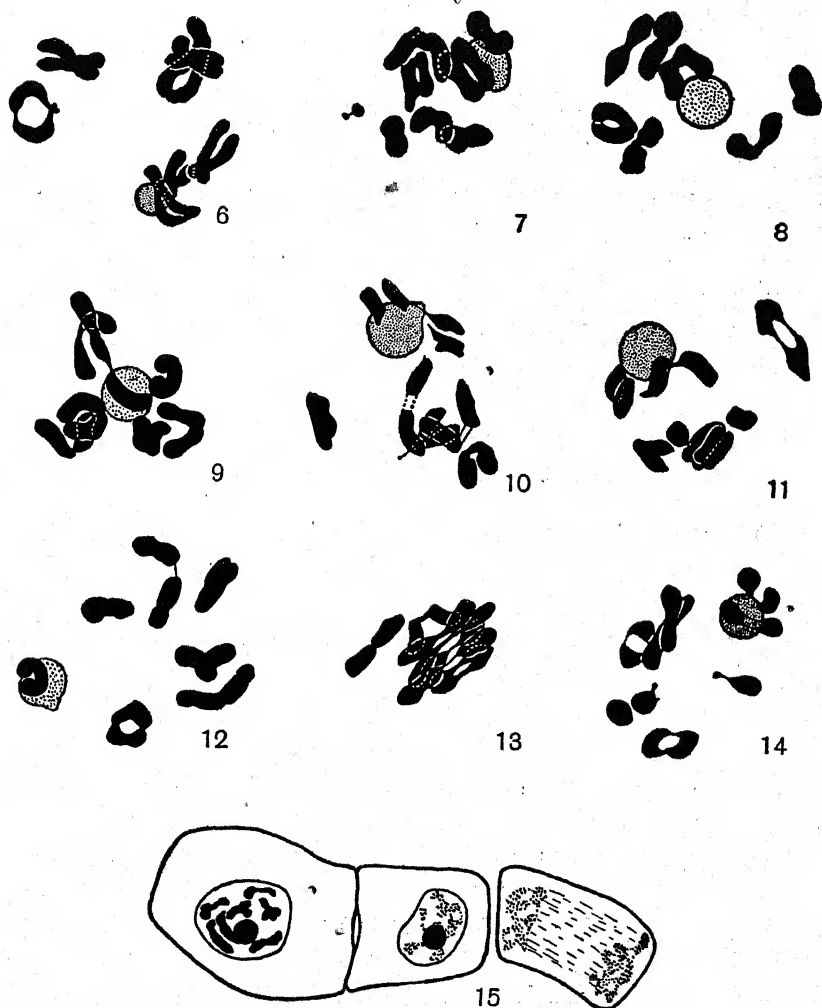
Text-figs. 6–15. Figs. 6–8, diakinesis and pro-metaphase in no-d. white plants. Figs. 9–13, diakinesis, pro-metaphase and metaphase in sulphur-white strains. Fig. 14, pro-metaphase in trisomic Crenate. Fig. 15, megaspore tetrad in a no-d. white strain. All except Fig. 14 are from Miss Saunders' material. Figs. 6–14, ca. × 3000. Fig. 15, ca. × 2000.

in its later meiotic phases. The fact that this is a plant of the variety
Snowflake, which has relatively uncontracted meiotic chromosomes,

may, on the other hand, b¦ significant. Similar constricted parts were found, but only very rarely, in aceto-carmine pollen mother-cell smears. They were seen oftener on fragmented chromosomes (see Text-figs. 42 and 43) than on whole ones. The fixation of the ovules seems quite good, and that of the aceto-carmine smears was excellent.

A study of the megaspore tetrads was made to see if any irregularities occurred in the determination of the megaspore which is to form the embryo-sac. In all cases clearly observable it was the cell at the chalazal end which formed the embryo-sac. A typical megaspore tetrad is shown in Text-fig. 15. The possibility of the micropylar cell occasionally forming the embryo-sac, as observed by Renner (1921) to occur regularly in certain Oenotheras, cannot, however, be entirely ruled out by the relatively few observations here made, for in accordance with the genetic calculations the maximum probability of its occurring would be about once in 6·7 times.

VI. Meiosis in pollen mother-cells of the short-chromosome types. (Miss Saunders' material.)

At the outset it may be mentioned that in the chromosomes of the pollen mother-cells of these types, there is no trace of the trabants which are seen in the somatic chromosomes.

Early prophase stages are very difficult to study in this material, but the configurations found at stages before diakinesis (Text-fig. 16) indicate a formation of chiasmata at random along the paired chromosomes. Later (as Darlington has shown in *Primula sinensis*, 1931 *a*) the association is found to be more and more terminal, so that at diakinesis it is almost entirely terminal. Although these associations cannot be critically diagnosed in all cases as chiasmata, the types of configuration agree in general with this interpretation, so that we may say that the metaphase end-to-end association is the result of terminalisation (more or less complete) of earlier interstitial chiasmata. There are certain special types of configuration which corroborate this conclusion. One is the survival to diakinesis and metaphase of identifiable interstitial chiasmata. A second is the occurrence of undoubted triple chiasmata with the characteristic triangular connection in the trisomic forms.

We shall therefore describe all connections between the chromosomes as chiasmata, a nomenclature which simplifies the description of the various configurations we are about to describe.

Twenty-three bivalent configurations taken at random from several

cells are illustrated in Text-fig. 16. These show the following chiasma frequency:

Chiasmata:	1	2	3	4
Bivalents:	3	12	7	1

The mean total chiasma frequency per bivalent at stages before diakinesis is 2·26, which is higher than at metaphase, 1·54, as would be expected. This variation is characteristic of that found for chiasmata by Maeda (1930) in *Vicia*, and Darlington (1930) in *Fritillaria*.
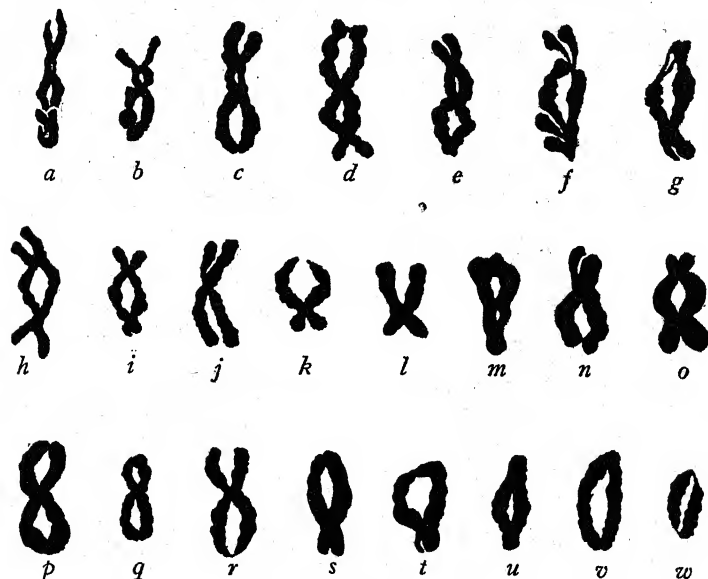
16

Text-fig. 16. Twenty-three bivalent configurations at stages prior to diakinesis. *a* has four chiasmata; *b–e*, *m*, *p* and *q* have three chiasmata; *f–i*, *n*, *o* and *r–w* have two chiasmata; *j–l* have one chiasma. (×4400.)

At diakinesis the 14 chromosomes are arranged in seven pairs. Commonly three or four ring bivalents are observed at this stage as a result of the maintenance of two terminal chiasmata. In the remaining bivalents the chromosomes are usually associated at one end only, owing to the retention of a single terminal chiasma (Text-figs. 17–20). Occasionally one bivalent shows a configuration which is interpreted as having one terminal and one sub-terminal chiasma (Text-figs. 19 and 20). In Text-fig. 19 one bivalent is seen to have its members widely separated, and connected by two fine threads. Only on one occasion has a fragment been observed at diakinesis (Text-fig. 18).

In side view metaphase two to five ring bivalents may occur. Two of these are characteristic and can almost always be distinguished. From their length, and from the median position of the point of spindle fibre attachment, it is probable that they are the chromosomes B and C. Their regularity is to be expected if terminalisation is, as Darlington suggests, away from the attachment constriction.
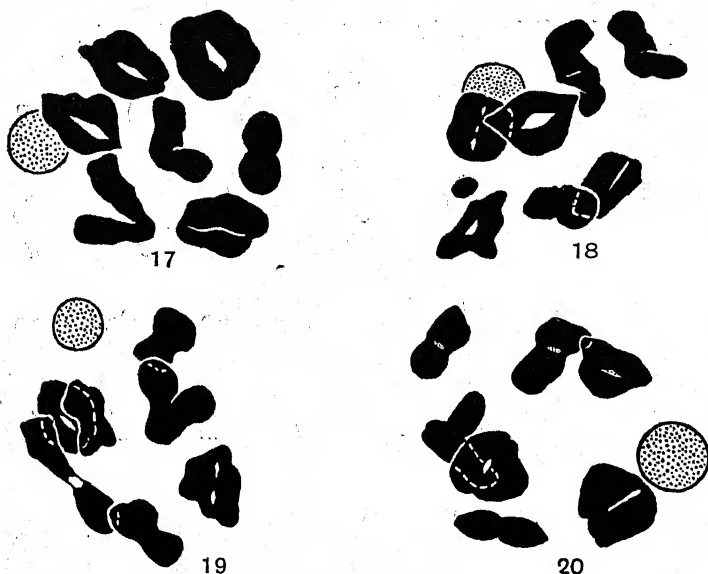
Text-figs. 17–20. Diakinesis. 17 and 18, no-d. race. 19 and 20, d. race. (×4400.)
Fig. 17. Four ring bivalents and three rod bivalents.
Fig. 18. Four ring bivalents, three rod bivalents and one fragment.
Fig. 19. Two ring bivalents, four rod bivalents and one bivalent having one terminal and one sub-terminal chiasma. One rod bivalent has its members widely separated and connected by two fine strands.
Fig. 20. Five ring bivalents, one rod bivalent and one bivalent having one terminal and one sub-terminal chiasma.

One bivalent frequently, and two bivalents less frequently, are observed to lie off the metaphase plate. This observation is most common in the d. races. Unpaired chromosomes have also been seen at this stage in the d. races.

In two strains, d. white and d. cream, bivalents have been observed to be attached to one another at early metaphase and at metaphase. In two cases six chromosomes, and in four cases four chromosomes are associated. Like the ordinary bivalent associations, these were always terminal, giving a string (Text-figs. 22–26) or ring (Text-fig. 27). Once, instead of the simple terminal chiasma found where only bivalents occur,

Text-fig. 21. Side view of first metaphase (no-d. race) showing four ring bivalents and three rod bivalents. Two ring bivalents are lying off the plate. (×4400.)
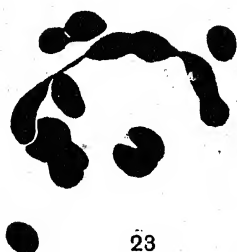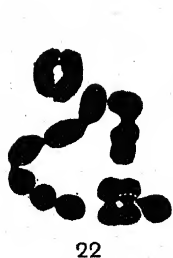
Text-figs. 22–27. First metaphase d. white and d. cream races. (×4400.)

Fig. 22. Early first metaphase. Six chromosomes associated end to end to form a string, two ring bivalents and two rod bivalents.

Fig. 23. Early first metaphase. Six chromosomes associated end to end, three of which are associated at one point, i.e. a triple chiasma. Three rod bivalents and two unpaired univalents.

Fig. 24. First metaphase. Four chromosomes associated end to end to form a string, two ring bivalents and three rod bivalents.

Fig. 25. First metaphase. Four chromosomes associated end to end to form a string, one ring bivalent and four rod bivalents.

Fig. 26. First metaphase, side view. Four chromosomes associated end to end to form a string, also one rod bivalent lying off the plate. Two ring bivalents and two rod bivalents lying on the plate.

Fig. 27. First metaphase, side view. Four chromosomes associated end to end to form a ring, also one rod bivalent lying off the plate. Four rod bivalents lying on the plate.

a triple chiasma was seen (cf. Darlington, 1929 *b* and *c*) where three chromosomes were associated terminally at one point (Text-fig. 23).

On the assumption that pairing of chromosomes at metaphase is determined by the association of homologous elements at prophase, the association of more than two chromosomes must be taken to show an abnormal constitution in the chromosome concerned. This constitution is fairly clear from the above observations. The ring or chain of four can be accounted for by the assumption of segmental interchange between the two non-homologous chromosomes of the pairs involved. This condition is illustrated diagrammatically by Darlington (1929 *a*) as the origin of a ring in *Oenothera*. Segmental interchange was first suggested by Belling and Blakeslee (1924, 1926) and by Belling (1927) to explain anomalous chromosome pairing in *Datura*, and has since been applied to *Oenothera* (Darlington, 1929 *a*), *Tradescantia, Rhoeo, Zebrina* (Darlington, 1929 *c*), *Aucuba* (Meurman, 1929), *Campanula* (Gairdner and Darlington, 1930) and Maize (Burnham, 1930). Cases described by Richardson (1929) and Håkansson (1929) in *Pisum*, by Kihara (1929) in *Humulus* and by Nishiyama (1929) in a triploid hybrid *Avena* are probably analogous.

The association of six is more complex, because the triple chiasma as found in one case shows that three chromosomes have corresponding elements. This diploid must therefore be trisomic (or possibly tetrasomic) in respect of a part of one chromosome. This reduplication would follow translocation. The fact that this configuration occurs rarely may indicate merely that the element in question is small, for Darlington (1929 *c* and 1930) has shown that small pieces of chromosome (fragments) pair rarely, although none the less homologous. This is particularly evident in the present case, for in the Stock, as has been pointed out, four or five of the chromosome pairs at metaphase are, as a rule, associated by only one chiasma. If this follows the terminalisation of interstitial chiasmata at diplotene (cf. Darlington, 1929), then, since the chiasma-frequency at metaphase in *Matthiola* is only 0·77 per whole chromosome, which is less than in *Campanula* (Gairdner and Darlington, 1930), elements of less than half one chromosome, though identical, cannot be expected to pair regularly. Size distinctions are difficult, and it has not been possible to determine whether the same bivalents are always involved in the formation of the quadrivalents and sexivalents. For the same reason it is also impossible to identify the bivalent with which the factors for singleness and doubleness are associated, but there is no doubt that structural changes have taken place in chromosomes other than this pair.

Early anaphase side view shows that the chromosomes are usually attached terminally (Text-figs. 28 and 29). Text-fig. 28 shows an exception in which the association of one pair is apparently by a sub-terminal chiasma, cf. diakinesis. One pair of chromosomes (probably the same pair as mentioned above) is frequently slower in dividing than the others, and in extreme cases may be still on the plate or stretched right across it when the other chromosomes are at the early telophase

28

29
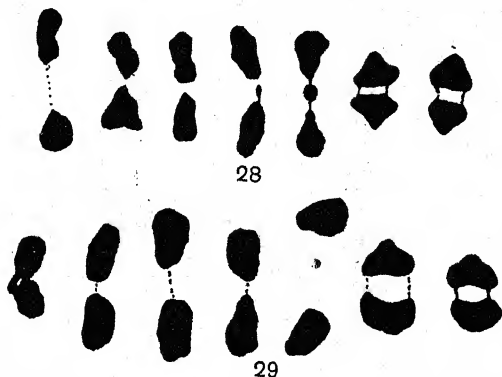
Text-figs. 28 and 29. Early first anaphase, side view, d. races. (× 4400.)
Fig. 28. All the chromosomes are terminally attached, with the exception of one pair which are sub-terminally attached. Note two characteristic ring bivalents almost always found at this stage.
Fig. 29. All the chromosomes terminally attached. Three ring bivalents and four rod bivalents.

stage. Various stages in the division of this bivalent are shown in Text-figs. 30–35. This behaviour is interpreted as being due to the incomplete terminalisation of the chiasmata at metaphase. This again may be due to the chromosome being of abnormal constitution at one end, the distal arms being of non-homologous segments. Such a conclusion is supported by the occurrence of quadrivalents.

Text-fig. 36 shows two daughter chromosomes, one at each pole, which lie well away from the rest of the chromosomes. This would be expected to result from the bivalent lying off the metaphase plate. Text-fig. 36 also shows, at the left of the plate, a laggard bivalent which is splitting.

The second division follows closely upon the first, and nearly always appears to be regular, although laggard chromosomes have been observed, and rather more frequently in the d. than in the no-d. races. A second-division metaphase is shown in Text-fig. 37.

Fragments have been observed in one plant of a no-d. race and in one plant of a d. race, at diakinesis (Text-fig. 18), anaphase (Text-fig. 35),

interkinesis (Text-fig. 38), and at the tetrad stage (Text-fig. 39). Darlington (1929 *a*) has expressed the opinion that fragmentation is usually associated with abnormalities in segmental homology. If this is so, then the discovery of fragments confirms the way in which the observations of multiple association of chromosomes have been interpreted. With the

Text-figs. 30–34. Late first anaphase, side views, d. races, showing the various stages in the separation of a lagging ring bivalent. 30 and 31 (× 4400). 32, 33 and 34 acetocarmine (× 3600).

Text-fig. 35. Late first anaphase to telophase (d. race). One lagging bivalent and one small fragment. (× 4400.)

exception of these two plants, however, only normal tetrads were found in both d. and no-d. races, in spite of all the irregularities mentioned above. This general regularity in tetrad formation is in agreement with observations made by Frost (1915).
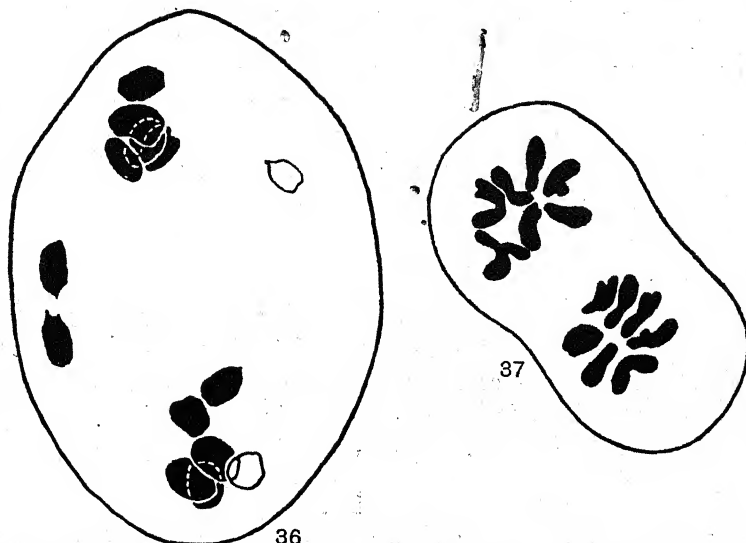
Text-fig. 36. First anaphase side view (d. race). Two daughter chromosomes (in outline) lying away from the rest of the chromosomes and a laggard bivalent which is splitting.
Text-fig. 37. Second division metaphase (d. race), showing seven chromosomes in each plate. Aceto-carmine. (× 4400.)
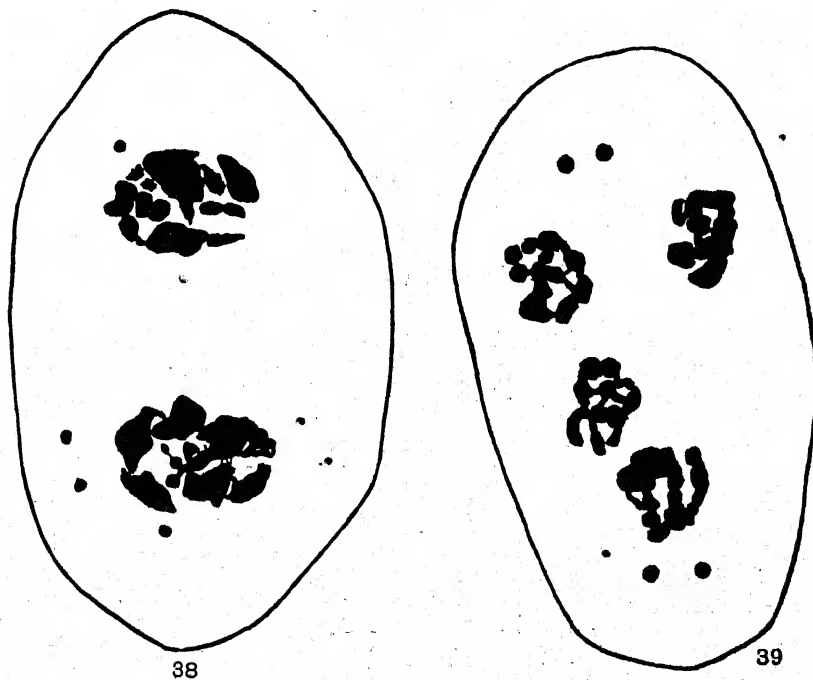
Text-fig. 38. Interkinesis (d. race) showing a fragment at each pole and two fragments which are lying nearer one plate.
Text-fig. 39. Early tetrad stage (d. race) showing two pairs of fragments which have probably arisen by equational division of two fragments at the second division; also one very small fragment.

The condition shows that we cannot expect to find a simple hypothesis to explain accurately all ratios found in the Stock. A part only of the structural hybridity of the Stock is the occurrence of the absence of the trabant to which we attribute a lethal effect in the phenomenon of "double-throwing."

VII. Meiosis in pollen mother-cells of the long-chromosome types.

The long-chromosome types of the variety Snowflake and its extra-chromosome mutants differ in a number of ways from the ordinary short-chromosome varieties. In the first place their meiotic chromosomes are very much easier to study. Details of their structure can be readily

TABLE I.

Chiasma frequency and position in long- and short-chromosome types of Matthiola incana *at first metaphase.*

Plant	Date fixed	No. of divisions	No. of bivalent chromosomes	Chiasmata per bivalent		Proportion interstitial
				Total	Interstitial	
Long chromosome strain, l_8	Jan. 1930	35	$\frac{490}{2} = 245$	1·55	0·19	0·12
Long chromosome strain, l_{15}	July 1930	35	$\frac{490}{2} = 245$	1·46	0·18	0·13
Short chromosome segregates, $18A_{1 \text{ and } 15}$	Jan. 1930	35	$\frac{490}{2} = 245$	1·62	0·04	0·03
Short chromosome strain, 32–7–3	July 1930	35	$\frac{490}{2} = 245$	1·47	0·05	0·04

Mean total chiasma frequency (long type) per bivalent = 1·50
or per chromosome = 0·75.
Mean total chiasma frequency (short type) per bivalent = 1·54
or per chromosome = 0·77.

observed, and some of the different types of chromosomes can be identified almost as well as in the somatic cells. At metaphase a striking difference is that whereas in the short-chromosome races the bivalents are practically always either rods or rings terminally connected, in the long-chromosome strains E type or key-ring bivalents are common. In terms of Darlington's (1929 c) chiasma theory of metaphase pairing, this means that in the former terminalisation of chiasmata is practically always complete before the metaphase stage is reached, whereas in the long-chromosome strains many of the chiasmata are still interstitial. Counts of terminal, interstitial and total chiasma frequency in short- and long-chromosome varieties and in short segregates from one of Frost's hybrids between short and long strains are given in Table I.

It will be seen that total chiasma frequency is slightly greater in the shorts, but that the proportion interstitial is much greater in the longs. The difference in total chiasma frequency is of doubtful significance; the difference in the interstitial frequency is clearly significant.

Text-figs. 40–47. Figs. 40–43 diploid var. Snowflake; Figs. 44–47 trisomic Crenate. See text for description. Aceto-carmine smears; Figs. 40–43 temporary and Figs. 44–47 permanent preparations. ca. ×3000.

Irregularities of meiosis are fairly common in the variety Snowflake. In Text-fig. 40 a cell is figured in which two unpaired chromosomes are lying on the central plate at metaphase. Three of the bivalents of this

cell are drawn so as to give an impression of the granular appearance of the chromosomes as seen in good permanent aceto-carmine preparations. Three others are drawn in outline only, so that they do not obscure the univalents which are lying in a plane above them. Text-fig. 41 shows an anaphase with two univalents lagging on or near the plate and beginning to divide. Bivalent chromosomes lying in irregular positions, such as along the metaphase plate at right angles to the other pairs, are frequently seen.

Fragmentation of chromosomes has been observed in a number of pollen mother-cells of long chromosome strains. In Text-figs. 42 and 43 it can be seen fairly definitely that the fragment has been broken off from the chromosome near which it is lying. In each case this is fairly definitely chromosome *A*, *i.e.* the chromosome carrying the doubleness-singleness factors. In Text-fig. 42 a thread is seen connecting the fragment to the middle of the chromosome. This seems to imply (cf. Darlington, *Tradescantia*, 1929 *c*) that there are homologous regions at the end and in the middle of this chromosome. The implication that segmental interchange or translocation has occurred is strengthened by the observation that one of the *A*-chromosomes alone can form a ring (cf. Belling, 1927). Some evidence for this was found in the diploid Snowflake variety, but the most conclusive cases occurred in Crenate plants (see Text-fig. 45). Apart from the more frequent observation of fragmentation, the abnormalities seen in the diploid Snowflake are therefore in general similar to those found in the short-chromosome races.

Crenate is one of Frost's mutant types which is trisomic for the *A*-chromosomes. The behaviour of the three chromosomes composing this trisome is of interest from several points of view, apart from doubling, and observations were therefore made in detail on 100 pollen mother-cells and the results tabulated with reference to the type of association and number of chiasmata of the trisome (see Table II).
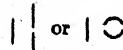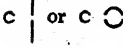
Rows 1-4 of Table II are configurations to be expected in a primary or secondary trisomic form, and rows 5 and 6 those found which are characteristic of a secondary trisomic, that is, a form in which the extra chromosome has some of its parts duplicated. Other secondary configurations which would be expected were not found, and it will be noted that secondary configurations have been found in only five cells. This is in agreement with expectation, since in the long-chromosome diploid form the chiasma frequency is only 0·75 per chromosome; and the reduplicated part being presumably only a small portion of the whole chromosome, it will be expected to be involved only occasionally in a

chiasma. The discovery of secondary configurations introduced complications which reduce the exact statistical value of the observations as a test of the chiasma theory of chromosome pairing, but do not affect their general agreement with expectation on that theory.

Crenate arises directly from the diploid Snowflake (cf. Frost, 1927). The clear evidence of duplication of parts within the A-chromosomes of Crenate therefore supports the cytological observations indicating duplication in the diploid Snowflake. Some of the configurations figured schematically in Table II are shown in Text-figs. 44–46. In Text-fig. 44 the trisomic group is arranged as a trivalent Y. Text-fig. 45 shows a

TABLE II.

Configurations and chiasma frequency of the trisome in Crenate Matthiola.

Arrangement of trisome	Minimum no. of chiasmata required at diplotene to give configuration	Times observed	Minimum chiasmata total
$|\ $ or $\|\ \bigcirc$	1·5*	22	33
$\|$ (three vertical strokes)	2	54	108
Y	2	17	34
$\|\ \bigcirc$	3	2	6
c $\|$ or c $\bigcirc$	2·5*	2	5
$\bigcirc$	3	3	9
		100	195

Mean minimum number of chiasmata per chromosome = 0·65.

* When one member of the trisome is unpaired it is not usually possible to determine whether the remaining two form a ring or are joined by only one chiasma. The mean of these two possibilities, which coincides with the mean chiasma frequency of 1·5 per bivalent found in the diploid form (Table I), is therefore assumed.

trivalent ring. Text-fig. 46 shows seven bivalent chromosomes and a univalent which has formed a ring. Two such cells were found. Text-fig. 47 shows an anaphase with one bivalent delayed in its division, similar to that shown in Text-figs. 30–35 which are from Miss Saunders' short-chromosome types. In Text-fig. 47 a separated trisome, presumably of A-chromosomes, can be distinguished, and one can therefore say fairly definitely that the laggard bivalent is *not* the one concerned in the transmission of doubling. A somewhat similar anaphase in Slender is shown in Text-fig. 63.

112 pollen tetrads of trisomic Crenate were examined to determine chromosome loss. The results are given in Table III and some of the types of tetrad found are shown in Text-figs. 48–53.

Only the 100 tetrads normal in general form were considered in counting chromosome loss. The remainder of the sample comprised six

TABLE III.

Pollen tetrads of trisomic Crenate Matthiola.

Normal: no chromosomes left out	One chromosome left out of microspore nuclei	Two chromosomes left out in one microspore	Two chromosomes left out in different pairs of of microspores	Two chromosomes left out in one pair of microspores	Three chromosomes left out
57	28	1	8	4	2

Text-figs. 48–53. Microspore "tetrads" of trisomic Crenate, ca. × 1500.

triads, including two with one microspore having a diploid nucleus, and four with one binucleate microspore, and six tetrads having one degenerated microspore. The chromosome loss in Crenate must be considered in connection with doubleness ratios of this strain.
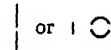
Slender *Matthiola*, as shown by Frost and Mann (1924) and Frost (1927, 1928), has a chromosome fragment which genetic analysis shows to be part of the *A*-chromosome and to carry doubleness-singleness factors. The configuration of this trisome was studied in 64 pollen mother-cells of Slender, with the result shown in Table IV.

Examples of the three trivalent configurations are shown in Text-figs. 54, 55 and 56.

The 64 cells observed included five which had a quadrivalent chromosome group. One of these clearly had a trivalent made up of two *A*'s and the fragmented *A*, in addition to the quadrivalent (Text-fig. 57).

TABLE IV.

Configuration and chiasma frequency of the trisome in Slender Matthiola.

Arrangement of trisome	Minimum no. of chiasmata required at diplotene	No. of times observed	Minimum total chiasmata	Minimum chiasmata for whole chromosomes	Minimum chiasmata for chromosome fragment
1. ǀ ǀ or ǀ ○	1·5*	43	64·5	64·5	0·0
2.	2	19	38·0	28·5	9·5
3.	2	2	6·0	4·0	2·0
4. ○	3	0	0·0	0·0	0·0
		64	108·5	97·0	11·5

Mean minimum chiasma frequency per whole chromosome $=\dfrac{97\cdot0}{64\times2}=0\cdot75$.

Mean minimum chiasma frequency per fragment chromosome $=\dfrac{11\cdot5}{64}=0\cdot18$.

* See footnote to Table II.

It is therefore evident that segmental interchange has occurred in chromosomes other than those concerned in the inheritance of doubleness. Text-fig. 58 shows an anaphase in which one bivalent is delayed in dividing, as in Text-fig. 47, and the trisome has segregated to give eight whole chromosomes at one pole and six and the fragment at the other. No secondary configurations were observed in Slender pollen mother-cells. However, in view of the fact that chromosome fragments do not pair as frequently as whole chromosomes, it is unsafe to draw the conclusion that the fragment is not duplicated in any of its parts, but it is a probability to be kept in mind in further genetic and cytological analysis of this material.

The relative frequency of trivalent formation in Crenate and Slender constitutes, despite the duplication, an excellent test of the chiasma theory of metaphase pairing. According to this theory the association of chromosomes at metaphase is a measure, not of a generalised attraction between them at this stage, but of the number of chiasmata or interchanges of chromatids that have occurred at the earlier stages of the prophase. On the older theory where metaphase pairing is the result of a general affinity of the whole chromosomes or whole chromatids, there are no clear reasons why a half or other fragment should not pair as regularly with a similar fragment, or with the whole chromosome from which it arose, as the original whole chromosomes did. On the chiasma theory, reduction in the length of the chromosome by fragmentation should reduce the number of chiasmata formed, and therefore reduce the frequency of pairing at metaphase. Crenate and Slender *Matthiola*, having respectively a whole extra *A*-chromosome and an extra fragment equal to about half the length of the whole *A*-chromosome, are especially favourable material for testing the chiasma hypothesis, despite the occurrence of cytological duplication in the *A*-chromosomes, since this duplication probably involves only a relatively small length of the chromosome, and its effect, if any, on chiasma formation will be the same in the diploid as in Crenate. In a comparison of Crenate and Slender if the duplication has any effect on chiasma frequency it will be to reduce it in the former.

Table II shows that an extra whole *A*-chromosome fails to pair with its homologues in 22 cases out of 100, whereas from Table IV we see that failure of pairing of the extra fragment of *A* occurred in 43 cases out of 64.

From Table I we find the average chiasma frequency for long-chromosome *Matthiola* to be about 1·5 per bivalent. We do not know the relative distribution of the chiasmata either in the different pairs or in different parts of the *A*-chromosome, but since the *A*-chromosome is one of the longer members of the set, it may be assumed to have somewhat more than the average frequency of 1·5 per bivalent. Since the paired lengths are the same at pachytene in diploids and triploids (cf. *Tulipa* and *Hyacinthus*), the chiasma frequency of the chromosomes comprising a trisome would be expected to be only about $\frac{2}{3}$ that of the same chromosomes in a diploid. Thus the expected chiasma frequency for the *A*-chromosome in Crenate would be somewhat more than 0·5. The frequency found is 0·65.

If the trisomic fragment of Slender has proportional chiasma frequency, then, being slightly more than half of the *A*-chromosome, the expectancy on the basis of the observed whole *A*-chromosome frequency

of 0·59 (Table IV) is slightly more than 0·29. But as Darlington (1930) has shown in *Fritillaria*, the pairing of fragments is slightly lower than would be expected simply from their length. This he attributes to a cumulative effect of prophase associations, one association helping the formation of another in adjacent lengths of chromosome. The chiasma frequency would therefore be expected to be reduced somewhat more than the chromosome length. In the trisomic fragment of Slender it would be expected to be less than 0·29. The maximum frequency found
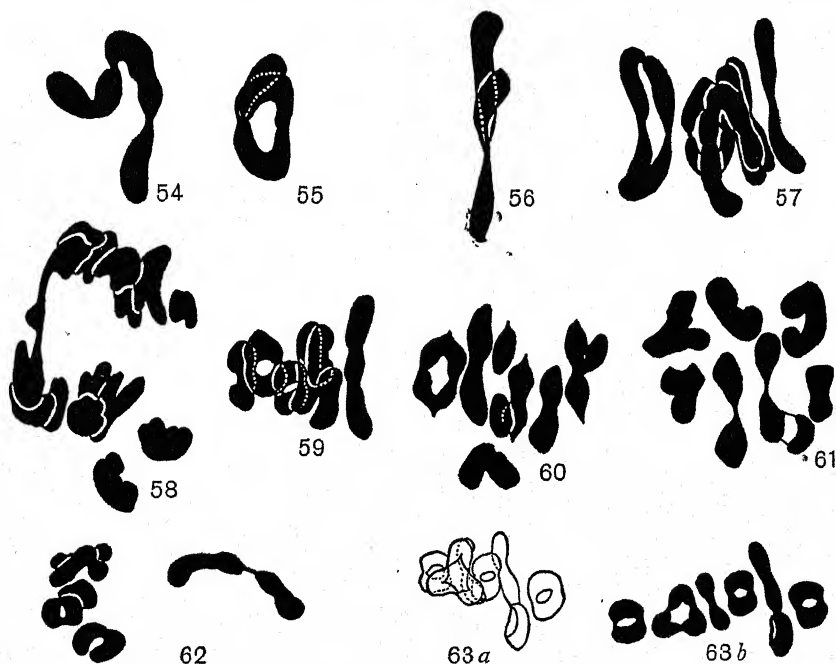
Text-figs. 54–63. Figs. 54–58 trisomic Slender. Figs. 59–63 *b* trisomic Smooth. See text for description. Figs. 54–61 permanent aceto-carmine smear preparations. Figs. 62 and 63 smears fixed in La Cour's "strong 2BE"—note smaller size. All ca. × 3000.
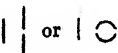
is 0·18. The results of this comparative study are therefore in accordance with the chiasma theory of metaphase pairing.

The cytological observations of frequency of trivalent formation, chromosome loss, etc. in Crenate and Slender are of importance in relation to their doubleness ratios. Since, however, comparatively little has yet been published on their genetics and Dr Frost informs us that he has many unpublished data, it seems advisable to present the cytological data independently and to leave them for the present uncorrelated with the few published genetical results.

Further evidence concerning the chiasma theory of pairing was sought in Smooth *Matthiola*, a trisomic form having an almost full-length extra chromosome. This is not the *A*-chromosome. Again, as in Crenate, trisomic configurations were found which require the assumption that it is a secondary trisomic, despite its primary origin. Further, configurations were found (see Text-figs. 59 and 63) in which the short chromosome was in the middle of a chain, or in a ring, and this suggests that it is this extra chromosome fragment which has duplicated parts.

<div align="center">TABLE V.</div>

Configurations and chiasma frequency of the trisome in Smooth Matthiola.

Arrangement of trisome	Minimum no. of chiasmata required at diplotene	No. of times observed	Minimum total chiasmata	Minimum chiasmata for whole chromosomes	Minimum chiasmata for chromosome fragment
	Primary configurations				
1. | | or | ◯	1·5*	15	22·5	22·5	0·0
2.	2	6	12·0	9·0	3·0
3.	2	1	2·0	1·0	1·0
4.	2	7	14·0	10·5	3·5
	Secondary configurations				
5.	3	3	9·0	6·0	3·0
6.	3	1	3·0	2·0	1·0
7.	3	2	6·0	4·0	2·0
		35	68·5	55·0	13·5

Mean minimum chiasma frequency per whole chromosome $= \dfrac{55·0}{35 \times 2} = 0·78$.

Mean minimum chiasma frequency per fragmented chromosome $= \dfrac{13·5}{35} = 0·39$.

<div align="center">* See footnote to Table II.</div>

Table V shows the arrangement and chiasma frequency in Smooth *Matthiola*. The formation of a trivalent occurs more frequently than in Slender. The reduction in chiasma frequency in the fragment is again slightly greater than would be expected from a consideration of their

and part chromosomes is difficult in some cases, as one cannot always determine, for instance in types 4 and 5, whether the fragment is attached by a chiasma to both chromosomes or only to one. Where there is doubt, it seems more in accordance with probability to credit the fragment with the minimum only, and this has been done throughout. The result from Smooth again seems to provide very good evidence in favour of the chiasma theory.

Various trivalent configurations found in Smooth are shown in Text-figs. 59 and 63. Text-fig. 59 shows a chain trivalent with the extra fragmented chromosome in the middle of the chain (type 3 of Table V). Text-fig. 60 shows a Y-shaped trivalent (type 4) and Text-fig. 61 a trivalent in which one end of the fragmented chromosome is attached to one of the whole chromosomes by a terminal chiasma at one end and by an interstitial chiasma at the other. Text-fig. 62 shows a quadrivalent lying far off the metaphase plate. The trivalent in this cell is lying close to a bivalent and its shape cannot be absolutely determined, but it is probably like that of Text-fig. 61. Text-fig. 63 a and b shows both a ring trivalent and a quadrivalent. In Text-fig. 63 a the chromosomes are drawn in outline in their actual position, and in Text-fig. 63 b they have been spaced out in drawing to show the separate chromosomes better. Text-figs. 62 and 63 obviously show further evidence of segmental duplication.

VIII. The nature of the mutation producing long meiotic chromosomes.

The variety Snowflake differs not only from all other known varieties of *Matthiola incana*, but also from all other members of the Cruciferae yet studied, in having very long chromosomes at the meiotic metaphase, as Lesley and Frost have shown (1927). It differs also from other varieties of *Matthiola* in that it throws a considerable proportion of chromosomally aberrant forms (see Frost, 1927). As has been shown above, the long- and short-chromosome types differ in the degree of terminalisation of their chiasmata before metaphase. Unpaired chromosomes and fragments occur frequently in the long-chromosome types, and only very rarely in the short types. The degree of meiotic contraction is regular in the short types, but varies considerably in different cells of the long type, and even within the same cell in rare cases. Incidentally, it may be mentioned that the long-chromosome types are very favourable material for studying the details of chromosome structure, chiasma nature and frequency at metaphase, etc., whereas the short-chromosome types are less favourable for such observations. Lesley and Frost (1927) have shown

that a single factor governs the difference between these types. The question naturally arises, What sort of factorial change might be expected to produce such a result?

Darlington (1931 *b*) has formulated an hypothesis which seeks to show the relationship between meiosis and mitosis. The central feature of this hypothesis is that in meiosis contraction of the chromosomes precedes their longitudinal splitting, instead of following it as in mitosis. If at a given stage of contraction there is an attraction between undivided chromosome threads in pairs, then in meiosis the attraction will be between whole chromosomes and in mitosis between longitudinal halves of chromosomes. The characteristic differences of meiosis and mitosis then follow logically, especially if one accepts Darlington's earlier (1929 *c*) hypothesis, that metaphase pairing is the resultant of prophase chiasma formation—an hypothesis which receives support from the observations recorded in the preceding section of this paper.

If we accept these hypotheses, it is interesting to note that all the differences between the long- and the short-chromosome types of *Matthiola* would follow if the mutation which produced the long type were one which merely delayed very slightly the onset of prophase contraction. Thus, splitting of single chromosomes would have begun, and pairing of whole chromosomes and chiasma formation would occur, not along the whole length of the chromosomes, but only along unsplit regions. In some chromosomes splitting might have proceeded to such a degree that insufficient chiasmata would be formed to maintain the association as bivalents to the metaphase, and unpaired chromosomes would be found at this stage. In all the chromosomes the average frequency of chiasmata formation would be lowered, but on the other hand the chiasmata would, provided the total time required for cell division remains unchanged, have less time in which to terminalise. The total chiasma frequency at metaphase might be the same in both types, owing to the two counter agencies of lower initial formation and less terminalisation in the long types; but, if so, the latter would certainly have a higher proportion of interstitial chiasmata. That these conditions are fulfilled is shown in Table I. The long type would obviously have longer chromosomes at metaphase merely because the chromosomes had had less time to contract—their condition being intermediate between that of normal meiotic and mitotic chromosomes. The occurrence of irregularity in the degree of contraction in the long type, while not evidence for the hypothesis, is nevertheless in accordance with it, since one might anticipate some irregularity in a mutation affecting such a delicate mechanism.

IX. Correlation of cytological evidence with the genetics of doubleness and plastid colour.

The inheritance of plastid colour and doubleness in Stocks is explained by Saunders (1911, 1916, 1928) on the basis of three linked factors **X**, **Y** and **W**. Both **X** and **Y** are necessary for singleness and **W** is the factor for white plastids, **w** being the recessive cream plastid factor. It is supposed that in the no-d. races **X** and **Y** are completely linked and crossing-over only takes place between $\overparen{\text{XY}}$ and **W**, while in the d.-throwing races **X** and **W** are completely linked and crossing-over only takes place between $\overparen{\text{WX}}$ and **Y**. This linkage does not affect the recessives. Pollen can only carry **X** and **Y** if they are linked; this can be taken simply as a fact determining the viability of pollen, or as resulting from pre-maturation segregation if that theory be accepted. This hypothesis, although it fits the observed results fairly well, is unorthodox, and does not explain the absence of particular classes of pollen. For these latter reasons Haldane and Waddington have each formulated a hypothesis of a more orthodox type, in which lethal factors are introduced.

Haldane (see Waddington, 1929) assumed that the recessives of two factors **P** and **O** were lethals; **p** was lethal to pollen containing it and the combination **op** was lethal to ovules. **P** was supposed to be very closely linked to the factor **S** for singleness and **O** closely linked to **W**. There was normal segregation of factors at gametogenesis, and crossing-over was almost entirely between **SP** and **OW** and only in ovule formation. This scheme, however, does not explain two later observations: first, that crossing-over between the factors for singleness and plastid colour has definitely been shown to take place in the formation of pollen in certain plants; and, secondly, that on crossing a d. white female with a no-d. cream male a small class of whites can be found in the F_1 which when selfed breeds true for singleness, but when crossed with a d. cream as male gives one white double to one cream single.

Waddington therefore produced a modified form of Haldane's hypothesis in order to explain the results. Since the second case arises in the discussion following the presentation of the hypothesis put forward in this paper, Waddington's scheme will be considered at that point.

Other hypotheses involving lethal factors have been put forward by Goldschmidt (1913), Frost (1915), Muller (1918); and as early as 1902 Correns had suggested that gametic elimination occurred in this material. Before considering the hypothesis put forward here, it must be noted that

except for the fact that the idea of selective elimination has been correlated with cytological observation and that the implications have been worked out in full, the hypothesis is essentially the same as that put forward by Frost in 1915.

The present hypothesis is based essentially upon evidence from the somatic chromosomes—the discovery that the A-chromosomes are those concerned with doubleness and that certain races are dimorphic in respect of these A-chromosomes. Pure singles were found to have a normal chromosome complement, the two A-chromosomes each having a trabant. Ever-sporting singles give rise to doubles and ever-sporting singles. These doubles also have a normal chromosome complement like the pure singles, but the ever-sporting singles have one A-chromosome which has no trabant, *i.e.* a deficiency in one A-chromosome. This deficiency will later be referred to as l.

It is supposed that singleness and doubleness are due to a single factor difference, singleness (**S**) being dominant to doubleness (**s**). Similarly colourless plastids (white) and cream plastids (cream) are due to a single factor difference, white (**W**) being dominant to cream (**w**). Since a linkage exists between the factors for singleness and plastid colour, both of these factors must be carried by the A-chromosomes. The factor **S** is not in the trabant, but is situated closer to it than is the factor **W**; so close that crossing-over never takes place between **S** and the trabant.

In the ever-sporting single it is supposed that the normal A-chromosome carries the recessive factor **s** for doubleness and also one of the factors for plastid colour, **W** or **w**. The A-chromosome with the deficiency, on the other hand, carries the dominant factor **S** for singleness and one of the factors for plastid colour.

On the male side the deficiency l is regarded as being lethal to gametes containing it, in so far as it inhibits fertilisation by these gametes. Further, in the formation of male gametes it prevents crossing-over between the two A-chromosomes, or, should crossing-over take place, it inhibits fertilisation by the two types of gametes thus produced. This would be the condition were part of the region between them inverted. Fertilisation therefore can only be effected by pollen containing the normal non-cross-over A-chromosome which carries the recessive factor **s** for doubleness. This is in agreement with our knowledge of the ever-sporting single when utilised as a male.

On the female side crossing-over is to take place at a point between the loci of **S** and **W**. From the breeding results it must be assumed that those female gametes which receive the cross-over A-chromosome with

the deficiency do not function, and it is also assumed that there is a partial elimination of female gametes containing the non-cross-over A-chromosome with the deficiency. The deficiency l is thus considered to be selective in its effect, being complete on the male side and incomplete on the female side.

In the pure single it is assumed that each of the two normal A-chromosomes carries the dominant factor **S** for singleness. There being no lethal, it is considered that crossing-over takes place in the normal way on both the male and female sides between the factors **S** and **W**, and that there is no elimination of any particular type of gamete. This also applies to any hybrid which has a normal chromosome complement.

The cross-over value between **S** and **W** is regarded as being constant for ever-sporting singles, pure singles and hybrids.

Table VI shows, for each mating, the expected proportions as percentages in accordance with Saunders', Waddington's and the present hypothesis, followed by the observed results (where they have been obtained) and the results to be expected as calculated on the present hypothesis. The observed results are those published by Miss Saunders (1928 and earlier). In addition, Miss Saunders has kindly provided us with previously unpublished results obtained on selfing a d. white and a d. cream. These had not been published because the proportion of singles and doubles was in agreement with that obtained on selfing other d.-throwing coloured races. They are included in this table, since they are of interest with regard to the assumption that there is a selective elimination of certain types of gametes.

The expected proportions have been calculated by Saunders and Waddington on a cross-over value of 6·25 per cent., but the cross-over value 3·75 per cent. (calculated from the sum of the observed results derived from the F_2 of matings (2a) and (3)) has been adopted in arriving at the present expected proportions. The percentage elimination of non-cross-over female gametes containing the deficiency was calculated from the observed results obtained from selfing and back-crossing the hybrids of matings (1) and (5). In mating (1) the hybrid is homozygous for singleness and there is 8 per cent. of elimination, whereas in mating (5), where the hybrid is heterozygous for singleness and is equivalent to an ever-sporting single, there is 31 per cent. elimination. It appears, therefore, that the elimination is also affected by the constitution of the plant in respect of the factors for singleness and doubleness.

It is important that special attention should be paid to this difference in elimination of the non-cross-over female gametes containing l in the

TABLE VI.

Constitution of hybrids and proportions in resulting families.

(A) Proportion expected on Saunders' hypothesis.
(B) Proportion expected on Waddington's hypothesis.
(C) Proportion expected on the present hypothesis.
(D) Observed proportions.
(E) Expected proportions calculated on the present hypothesis.

Cross	F_1 plants		F_2 from F_1 selfed				F_2 from F_1 × d. cream				F_2 from d. cream × F_1			
			White single	Cream single	White double	Cream double	White single	Cream single	White double	Cream double	White single	Cream single	White double	Cream double
(1) d. white × no d. cream; sulphur-white × no d. cream	$\dfrac{\text{WSl}}{\text{wSL}}$	(A)	50	50	0	0	50	50	0	0	0	100	0	0
		(B)	51·6	48·4	0	0	51·6	48·4	0	0	0	100	0	0
		(C)	48·9	51·1	0	0	48·9	51·1	0	0	0	100	0	0
		(D)	186	192	0	0	102	108	0	0	0	all	—	0
		(E)	185·1	193·1	0	0	102·8	107·3	0	0	—	—	—	—
(2) d. white × no. d. cream	$\dfrac{\text{WsL}}{\text{wSL}}$	(A)	50·1	24·9	24·9	0·1	3·1	46·9	46·9	3·1	25·1	48·3	24·9	1·7
		(B)	50·1	24·9	24·9	0·1	3·1	46·9	46·9	3·1	25·8	48·4	24·2	1·6
		(C)	50·0	25·0	25·0	0·03	1·9	48·1	48·1	1·9	21·1	48·9	28·9	1·1
		(D)	110	39	57	0	21	277	328	7	107	213	127	7
		(E)	103·0	51·4	51·4	0·72	11·9	304·7	304·7	11·9	95·7	221·9	131·3	5·1
						$P=0.1901$				$P=0.0041$				$P=0.4752$
d. cream × no d. white	$\dfrac{\text{wSl}}{\text{WSL}}$	(A)	100	0	0	0	50	50	0	0	100	0	0	0
		(B)	100	0	0	0	48·4	51·6	0	0	100	0	0	0
		(C)	100	0	0	0	51·1	48·9	0	0	100	0	0	0
		(D)	all	0	0	0	—	—	—	—	—	—	—	—
(3) d. cream × no d. white; sulphur-white × no d. white	$\dfrac{\text{wsL}}{\text{WSL}}$	(A)	72·0	3·0	3·0	22·0	46·9	3·1	3·1	46·9	48·3	25·1	1·7	24·9
		(B)	72·0	3·0	3·0	22·0	46·9	3·1	3·1	46·9	46·6	25·9	3·1	24·3
		(C)	73·2	1·8	1·8	23·2	48·1	1·9	1·9	48·1	48·9	21·1	1·1	28·9
		(D)	1302	27	36	331	853	31	34	844	79	39	1	47
		(E)	1241	31·2	31·2	392·9	848·0	33·0	33·0	848·0	81·1	35·0	1·9	48·0
						$P=0.0031$				$P=0.9796$				$P=0.8147$
No d. white × d. cream	$\dfrac{\text{WSL}}{\text{wsL}}$	(A)	72·0	3·0	3·0	22·0	46·9	3·1	3·1	46·9	48·3	25·1	1·7	24·9
		(B)	72·0	3·0	3·0	22·0	46·9	3·1	3·1	46·9	46·6	25·9	3·1	24·3
		(C)	73·2	1·8	1·8	23·2	48·1	1·9	1·9	48·1	48·9	21·1	1·1	28·9
		(D)	1008	25	25	349	853	31	34	844	75	41	3	40
		(E)	1029	25·9	25·9	325·8	848·0	33·0	33·0	848·0	77·7	33·5	1·8	46·0
						$P=0.5299$				$P=0.9796$				$P=0.3506$

Genetics data table (stocks — Matthiola), matings 4–9 with expected/observed segregation ratios. Sub-rows: (A)(B)(C) = expected percentages, (D) = observed numbers, (E) = expected numbers, with χ² probability (P).

Mating	Genotype														
(4) No d. cream × d. white	wSL/WsL (A)	50·1	24·9	24·9	0·1	3·1	46·9	46·9	3·1	25·1	48·3	24·9	1·7		
	(B)	50·1	24·9	24·9	0·1	3·1	46·9	46·9	3·1	25·8	48·4	24·2	1·6		
	(C)	50·0	25·0	25·0	0·03	1·9	48·1	48·1	1·9	21·1	48·9	28·9	1·1		
	(D)	3195	1255	1509	3	4	25	39	0	124	302	179	13		
	(E)	2983	1488	1488	2·1	1·3	32·7	32·7	1·3	130·2	302·0	178·7	7·0		
			P=0·0000					P=0·0180					P=0·3872		
(5) d. white × d. cream / sulphur-white × d. cream	WSl/wsL (A)	46·9	3·1	3·1	46·9	3·1	3·1	46·9	50	46·9	46·9	3·1	53·1		
	(B)	48·4	3·2	3·2	48·4	3·2	3·2	48·4	48·4	48·4	48·4	3·2	51·6		
	(C)	39·9	2·3	2·3	39·9	2·3	2·3	57·9	57·9	39·9	39·9	2·3	60·1		
	(D)	170*	10						253	80		10	118		
	(E)	172·8	9·8						250	79·0		9·8	119·1		
(6) d. cream × d. white	wSl/WsL (A)	46·9	53·1	46·9	46·9	3·1	46·9	46·9		53·1					
	(B)	48·4	51·6	48·4	48·4	3·2	48·4	48·4		51·6					
	(C)	39·9	60·1	39·9	57·9	2·3	39·9	60·1		60·1					
	(D)						9	8·8							
	(E)				0·5		8·8	12·7							
								P=0·7753							
(7) d. white × no d. cream / sulphur-white × no d. cream	WSl/wSL (A)	50	50	0	0	0	50	50	100						
	(B)	50	50	0	0	0	50	50	100						
	(C)	48·9	51·1	48·9	0	0	0	51·1	100						
	(D)			0			180	171							
(8) d. white selfed	WSl/WsM / TsM (A)	46·9	0	0	0	0									
	(B)	48·4	0	0	0	0									
	(C)	39·9	0	57·9	0	0									
	(D)	522	0	753											
	(E)	508·9	0	766·3											
		P=0·4685													
(9) d. cream selfed	lSw/Tsw (A)	0	0	0	0					53·1					
	(B)	0	0	0	0					51·6					
	(C)	0	0	0	0					60·1					
	(D)	0	496	0	0					687					
	(E)	0	472·2	0	0					711·1					
			P=0·1715												

* The observed results are actually the F₃ ex (F₂ selfed) of this mating, but Saunders states that the F₁ gave the same proportions.

two types of plant. Although there is no cytological evidence to show that there is a mechanism in action comparable to that found by Renner (1921) in *Oenothera*, such a hypothesis would explain very well the assumption of selective elimination of certain female gametes. Thus in one race $\frac{Sl}{sL}$ there is 31 per cent. elimination of non-cross-over female gametes containing **Sl**, while in another race $\frac{Sl}{SL}$ there is 8 per cent. elimination of non-cross-over female gametes **Sl**. It may be considered that the **Sl** complex is weakly active in relation to its partner complex in the development of the embryo-sac, while the complex **sL** is strongly active, and the complex **SL** is of medium activity. Such a form of developmental selection taking place between the different complexes in the ovules as a result of the competition between the megaspores would therefore give rise to an apparent selective elimination, in different proportions in different zygotes, of the **Sl** ovules. A further advantage of such a hypothesis is that it does not require the production of a constant proportion of aborted ovules or inviable seeds by the ever-sporting singles.

As an alternative to the "Renner Effect" we must assume that the deficiency l has a weakening effect on the ovules containing it, so that a practically constant proportion of these ovules does not eventually produce viable seed.

The hypothesis now having been presented, we will consider the possible objections to it. First, we have no proof that the cross-over female gametes which are eliminated do carry l. It cannot be proved cytologically that these ovules are carrying the chromosome which has lost a trabant, but it is possible to prove this in an indirect way by examination of the somatic chromosomes of the cross-over double white derived from a sulphur-white. Such a plant was found to have two *A*-chromosomes each bearing a trabant, and therefore the logical conclusion is that the non-functioning cross-over class is carrying the lethal l. Secondly, there is no direct evidence to show that there is elimination of certain classes of ovules. It seems, however, from the genetical results that some sort of elimination is taking place. The difference in elimination according to the constitution of the plants points very strongly towards a Renner effect as being the explanation, but cytological evidence would be required to substantiate this. The alternative to the Renner effect, that the deficiency l has a weakening effect on the ovules, has no exact parallel. In aneuploid *Matthiola* (Frost and Mann, 1924, and Frost, 1919,

1927), *Datura* (Blakeslee, 1921, and Blakeslee and Belling, 1924), *Solanum* (Lesley, 1928) and *Oenothera* (De Vries and Boedijn, 1923) aneuploid male gametes are however almost entirely non-viable and only a proportion of aneuploid female gametes produce viable seeds. The above assumption therefore, seems quite reasonable in view of the effect of 1 in the male gametes. On this basis it follows that ever-sporting singles should produce a more or less constant proportion of either undeveloped ovules, undeveloped seeds or inviable seeds. Saunders (1916), however, in criticising Frost's hypothesis, stated that she had found a full complement of seeds set as a result of natural fertilisation in both pure singles and ever-sporting singles, and that she had no evidence of a constant proportion of inviable seeds.

Table VII shows the results of a few counts which have been made of the seed production in a few samples of pods, selected from plants grown out-of-doors and allowed to set natural seed. Six plants of the types

TABLE VII.

Analysis of seed production.

	Pods	Good seeds	Bad seeds	% bad seeds	S.D.
Sulphur-white	12	414	127	23·47	±12·26
"	12	480	93	16·23	±15·62
D. cream	9	359	71	16·51	±15·13
No d. white	12	416	37	8·16	± 8·73
No d. cream	9	341	99	22·5	±21·71
No d. red	12	428	14	3·16	± 3·53

shown were selected at random and a number of pods taken, also at random, from each. The numbers of good and bad seed were counted separately for each loculus, hence two sets of results were obtained for each pod.

Although the table shows the sum of the results obtained from each plant, the standard deviation was derived from the percentage of bad seeds per loculus, and is therefore much more sensitive than if it had been obtained by utilising the percentage of bad seeds per plant as shown in the table. These results, although the numbers are not large, show that seed production is by no means constant in all cases and that in some cases there is a definite elimination of seed.

A discussion of the implications of certain cytological evidence on these observations will be made at a later point. This will show that the assumption of a Renner effect, or simply partial elimination, cannot be disproved until much further work is carried out.

Since the sulphur-white never gives rise to the cross-over cream single,

it is assumed that the female gametes containing the cross-over A-chromosome carrying l are totally eliminated. Only partial elimination of female gametes containing the non-cross-over A carrying l however is assumed to take place. This is assuming a new type of phenomenon, and the only explanation which can be put forward at present is that the presence of l combined with some physical effect resulting from, or during, crossing-over causes complete elimination of these gametes.

Although in this hypothesis the assumption is made that there is total elimination of the cross-over female gametes containing l, it is possible that the elimination may be only partial, and to the same extent as in the non-cross-over female gametes containing l. On this basis, however, we should expect only one cream single in every 65·3 plants produced by selfing a sulphur-white, and Saunders' figures ($n = 433$) seem to disprove this possibility.

There is therefore on the whole no really serious objection to the hypothesis, and, apart from the fact that it involves only one lethal, it has the advantage over other hypotheses in that it is based primarily upon cytological evidence.

The application of the hypothesis (Table VI) will now be considered. It would appear that the most serious discrepancy occurs in the results obtained from crossing the F_1 white single out of the cross d. white × no-d. cream, with a d. cream as male (7). Saunders considers that this F_1 hybrid is a cross-over of the constitution $\dfrac{\widehat{WXy}}{\widehat{wXY}}$ or $\dfrac{\widehat{WxY}}{\widehat{wXY}}$. On crossing it with a d. cream as male it would give equal numbers of cream singles and white doubles and on selfing it would breed true for singleness. Waddington considers this hybrid to be of the constitution $\dfrac{VslW}{VSLw}$. L is supposed to be closely linked to W, and S is closely linked with the ovule factor V. l is always lethal to pollen and vL is lethal to both pollen and ovules, whilst vl is lethal to pollen only. Crossing-over is to take place both in the male and the female between VS and LW. In this particular case a special assumption has to be made in order to explain the non-production of white doubles on selfing this hybrid. Waddington assumes that the combination Vl suppresses crossing-over in both ovules and pollen.

In the present scheme, on crossing a d. white with a no-d. cream as male, only two types of white single are produced, for the two cross-over types are identical in constitution with the non-cross-over types. It does not therefore allow of a type of white single which on selfing will

breed true for singleness and which, on crossing with a d. cream as male, will give cream singles and white doubles. It is suggested that probably those plants which have the constitution $\dfrac{\text{WSl}}{\text{wSL}}$ (or Saunders' non-cross-over $\dfrac{\widehat{\text{WXY}}}{\text{w}\widehat{\text{XY}}}$) have been selfed, and that those plants which have the constitution $\dfrac{\text{WsL}}{\text{wSL}}$ (or Saunders' non-cross-over $\dfrac{\text{Wxy}}{\text{w}\widehat{\text{XY}}}$) have been utilised as females when crossed with a d. cream. According to the present scheme we should expect from this cross 3 white single, 77 cream single, 77 white double and 3 cream double plants. Saunders' observed results, 171 cream singles and 180 white doubles, are derived from eight different families and *no individual family numbered more than* 90 *plants.* Since we should expect only 1 white single and 1 cream double in every 53 plants, it is not surprising that a number of these families did not contain either of these two classes. On selfing a white single of the constitution $\dfrac{\text{WSl}}{\text{wSL}}$ it should breed true for singleness. The figures obtained as a result of selfing this supposed class of cross-over whites are not given, and Miss Saunders tells us that unfortunately one and the same plant has never been used for selfing and for crossing with a d. cream as male. Until this is done we have therefore no proof that such a class of white singles exists. The discrepancy as shown in Table VI (7) may thus be quite false.

The divergence between the observed results and the expected of the F_2's (2a) and (4) are not of great significance, for the F_2 (3) according to this scheme is derived from a hybrid of the same constitution as (2a) and the F_2 (1a) similarly corresponds to that of (4), and these agree with expectation. Further, these hybrids used as male and female when crossed with a d. cream give results which are also in agreement with expectation, with perhaps one exception, namely, the F_2 from the F_1 as female, crossed with a d. cream (1a).

In general the expected results fit the observed results remarkably well, and much better than those derived from Saunders' and Waddington's hypotheses. Explanations have been offered for the most striking disagreements, and it must be remembered that a certain amount of deviation is to be expected, primarily because most of the observed results are aggregates from different plants (the importance of this will be explained later) thus affecting the derived values for crossing-over and elimination, and because these values have been considered as being constant throughout.

DIAGRAMATIC EXPLANATION OF THE GENETIC BEHAVIOUR OF EVER-SPORTING SINGLE, PURE SINGLE AND HYBRID RACES OF STOCK.

Along with segmental interchange we have segmental reduplication giving rise to further diversity in the chromosome constitution of different plants. We should therefore have a very great range of plants which differ in the constitution of their chromosome complex in varying degrees. Some will be entirely stable while others will be unstable in respect of four or more of their chromosomes. Although plants such as these should exist and be fertile, they will produce heteroploid gametes, and also gametes containing the chromosome complement complete but rearranged. Sterility is associated with such abnormalities in *Datura* (Blakeslee, 1929) and in *Drosophila* (Muller, 1930) where the translocated piece is approximately half a chromosome, but, arguing on the same lines as before, viz. that the translocated or reduplicated segments are very small, the adverse effect on the viability of these gametes will certainly be variable; it will alter with the constitution of the chromosome complex, but in any case will be slight.

In view of these possibilities the different chromosome complexes associated with the deficiency 1 will play a part especially noticeable in the viability of the female gametes, and it may well be that the degree of elimination of the particular class of female gametes varies in the different strains, *i.e.* between pure singles and ever-sporting singles and also within these groups.

The fact that Saunders (1916) has found pods, both in pure-breeding and ever-sporting singles, which have set a full complement of seeds does not disprove the possibility of simple elimination of ovules (as opposed to a Renner effect). We should expect some variation in the proportion of viable seeds produced, the differences to be constant in some families and to vary in others, according to whether the chromosome constitution is stable or not. Table VII gives a few counts of the seed production in plants of different strains and, besides showing that there seems to be some elimination, tends to support this view. Kvasnikov (1929, p. 95), in a paper which reached us after the observations in this paper were completed, says that "pure-breeding single offspring flower early and are very fertile if compared with sister seed plants of the ever-sporting type, which develop slowly, have less flowers, a greater sterility and therefore less seed."

As a result of the variation in the degree of elimination of the ovules, we should expect some slight differences in the proportion of singles to doubles produced by different plants. In some cases where the chromosome constitution is stable the progeny would continue to give ratios like the parent. In others, different members of the progeny would give

different ratios as a result of the instability of the chromosome constitution of the parent plant.

Kvasnikov's further statement that pure-breeding singles arise as rare segregates from ever-sporting plants, if substantiated, indicates either that normal crossing-over can occur, though rarely, between the trabant, L and s, or that some irregular cytological change occurs to restore the trabant to the A-chromosome carrying singleness.

From the amount of cytological evidence so far obtained, it would be premature to attempt to explain the inheritance of doubleness in the ever-sporting single purely on the basis of segmental interchange or segmental reduplication. Much critical work would also be necessary, especially with regard to fertility, in order to obtain evidence which would substantiate the conclusions following from such a hypothesis.

There is no doubt however that structural hybridity can play an important part in the production of strains which differ in their ratios of singles and doubles, and it may even affect crossing-over. Since most of the observed results are aggregates from different plants, these possibilities cannot be taken into account in the application of the hypothesis. It is therefore only natural to expect some disagreement between the observed results and the expectation on this hypothesis, but the disagreement is less than that of other hypotheses.

XI. Summary.

1. Study of the somatic chromosomes has shown that the ever-sporting races of *Matthiola incana* have a heteromorphic pair of chromosomes ("A"), one of the members having lost a trabant. Pure singles and doubles have a full chromosome complement.

2. "Crenate," a race trisomic for doubleness-singleness factors (Frost), has three A-chromosomes, thus proving this chromosome to be the one concerned in the inheritance of doubleness.

3. Ovule studies have not revealed selective determination of the embryo-sac mother-cells.

4. Frost's variety "Snowflake" and its mutants have long meiotic chromosomes. All other races studied have short meiotic chromosomes.

5. Pollen mother-cell meiosis in the short chromosome races shows various irregularities, including multiple associations indicating segmental interchange and reduplication, but none have been directly correlated with the inheritance of doubleness.

6. Irregularities of a similar nature occur in the pollen mother-cells of the long chromosome strains, but more frequently.

7. Trisomic strains have been utilised to test Darlington's chiasma theory of metaphase pairing, and they provide strong evidence in its favour.

8. The mutation producing long chromosomes is correlated with other changes expected on Darlington's hypothesis, which seeks to explain the relationship of meiosis to mitosis.

9. The trabant deficiency in the *A*-chromosome is regarded as being completely lethal to male gametes and incompletely lethal to female gametes, thus giving the ever-sporting character to the single. The bearing of segmental interchange and reduplication is also considered. Expected proportions on this hypothesis provide a closer fit to the genetic observations than those based on previous hypotheses.

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THE CYTOLOGICAL THEORY OF INHERITANCE IN *OENOTHERA*.

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(With Plate XII and Twenty-nine Text-figures.)

CONTENTS

I. INTRODUCTION.

THE genetical and cytological methods of enquiry each have serious limitations, and in the hybrid *Oenothera* species to a greater extent than in other organisms, on account of the complexity of the hereditary mechanism. It is therefore particularly important in this group to establish the theoretical connection between the two methods on a sound basis.

As a beginning I have described a hypothesis (1929 *a*) which differed from other hypotheses in recognising that chromosome behaviour at meiosis in *Oenothera* is consistent with the most generally applicable principles. These principles I have since attempted to define (1931 *b*). The hypothesis also differed in that it could be made the basis of exact predictions. Since then the assumptions have been strengthened and the predictions tested in the following six ways.

First, in regard to chromosome configurations in hybrids between different species, such predictions have been made and verified by Cleland and Blakeslee (1930). Sheffield (1929) has also found chromosome configurations in *Oenothera* hybrids which are in accordance with the hypothesis. (This was ignored by Sheffield (*loc. cit.*) and Gates (1930) in criticising the hypothesis.) Secondly, in regard to chromosome configurations in polyploid forms, such predictions have been verified by Håkansson (1930 *b*) and Catcheside (1931). Thirdly, Muller (1930 *a* and *b*) has inferred segmental interchange in *Drosophila* and its effect on the segregation of the segments by a logical process entirely different from that by which it was originally inferred by Belling from his illuminating observations in *Datura* and applied in my hypothesis to *Oenothera*. Fourthly, the origin of ring-forming systems, like that in *Oenothera*, in normally paired species has been described in *Pisum* (Håkansson, 1928; Richardson, 1929), *Campanula* (Gairdner and Darlington, 1930), *Zea* (Burnham, 1930) and *Rosa* (Erlanson, 1931); while Kattermann (1931) has explained ring formation in diploid *Briza* and *Anthoxanthum* on the same assumptions. Fifthly, the origin of ring pairs, by terminalisation of chiasmata following synapsis, has since been described in several other plants (*vide* Section VII).

Finally, the hypothesis is founded on the observation, which we owe originally to Cleland (1922 *et seq.*), that the association of chromosomes at meiosis in *Oenothera* is specific and constant, and may therefore be supposed to depend on the specific and constant attraction of identical pairs of elements. This conclusion continues to be universally verified (Appendix II).

I shall now attempt to formulate a precise theory of chromosome behaviour in the structurally hybrid *Oenothera* species and show how this condition of structural hybridity can have arisen. Now that I have described the inductive basis of the hypothesis more fully (1931 *b*), and defined a working hypothesis with regard to the cytological basis of crossing over, I also hope to be able to show that more far-reaching conclusions can be arrived at; that these conclusions are consistent with the theory and, indeed, seem to follow inevitably from it (Sections VII–XII).

Prof. J. B. S. Haldane has added an appendix on the number of possible pairing types in *Oenothera* and the mathematical theory of ring formation in general.

II. METHODS.

I have endeavoured to obtain critical evidence of behaviour not described by earlier workers on *Oenothera* by employing the technique perfected in this laboratory, which consists in:

(i) fixation of separate anthers in medium Flemming or La Cour's (1929) fixative for twelve hours;

(ii) washing by changes of tepid water (ca. 30° C.) in the same bottle for two hours;

(iii) cutting embedded material at $20\,\mu$ (instead of $8\text{–}12\,\mu$);

(iv) staining by Newton's gentian-violet method (Newton and Darlington, 1929).

I am indebted to Mr La Cour for making the preparations by these methods which he has described elsewhere (1931) in detail.

Drawings were made at bench level with the aid of a Zeiss pointolite lamp, 1·5 mm. oil immersion objective (n.a. 1·3), × 30 compensating eyepiece and camera lucida with green Wratten filters. The magnification with a tube length of 145 mm. was 6400, and with a tube length of 190 mm. was 9600. Where necessary for clearness in side views of metaphase separate configurations have been drawn separately.

III. MATERIAL.

Seven individuals were examined:

(1) *Oenothera berteriana* ($2n$);

(2) *Oe. muricata* ($2n$);

(3) *Oe. albiflexa gigas* ($4n$);

(4) *Oe. Lamarckiana* ($3n+1$) × *Oe. albiflexa gigas* ($4n$);

(5) *Oe. biennis* ($2n$), two plants, A and B;

(6) *Oe. biennis* ($2n$), plant C.

Nos. 2, 3 and 4 were from seed kindly supplied by Prof. Renner. No. 5, A and B, were seedlings found growing wild at Camberley in Surrey, and no. 6 (plant C) was from seed labelled "*Oe. rubrinervis*" from the Botanic Garden, Glasgow.

Somatic mitoses were examined in nos. 1, 3, 4 and 5 A, and pollen mother-cells at diakinesis, first metaphase, and anaphase in the diploid forms nos. 2, 5 A and B and 6.

IV. SOMATIC CHROMOSOMES.

The somatic chromosomes of *Oenothera* have been frequently illustrated, but rather unsatisfactorily. The best figure is that given by Davis (1909, Fig. 6). I find that they show an unusual complexity of visible structure (Text-figs. 1–4; Plate XII, figs. 1–3). They differ in
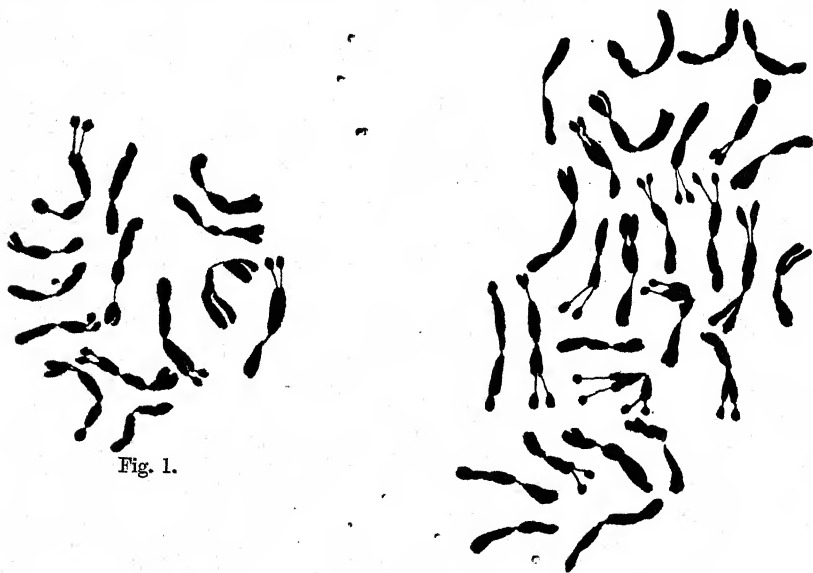
Text-figs. 1 and 2. Somatic mitoses. × 6400.
Fig. 1. *Oenothera berteriana*, 2n=14. Cf. microphotograph, Plate XII, figs. 1 and 2.
Fig. 2. *Oe. albiflexa gigas*, 2n=28.

length, the extremes being about 1·5 and 2·5 μ. The individuals examined were complex heterozygotes: their chromosomes cannot be paired in the diploid or arranged in threes in the triploid. This is in accordance with the hypothesis that they are structural hybrids. It is interesting to notice (Text-fig. 5) a similar complexity in the chromosomes of a non-

hybrid *Datura* species, where, however, the chromosomes can probably be paired (cf. Levitsky, 1929). The occurrence of marked constrictions in the chromosomes probably accounts for the observations of Lutz (1916) and Hance (1918) of supernumerary chromosomes in *Oenothera* (cf. microphotographs).
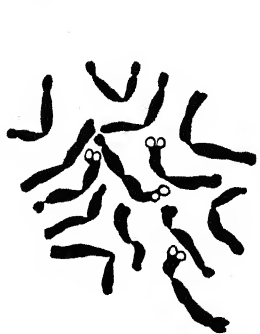
Fig. 3. Fig. 4.

Text-figs. 3 and 4. Somatic mitoses. × 6400.

Fig. 3. *Oe. biennis*, A, 2n = 14. [From an anther wall: inferior fixation.]
Fig. 4. *Oe. Lamarckiana* × *albiflexa gigas*, 2n = 22.
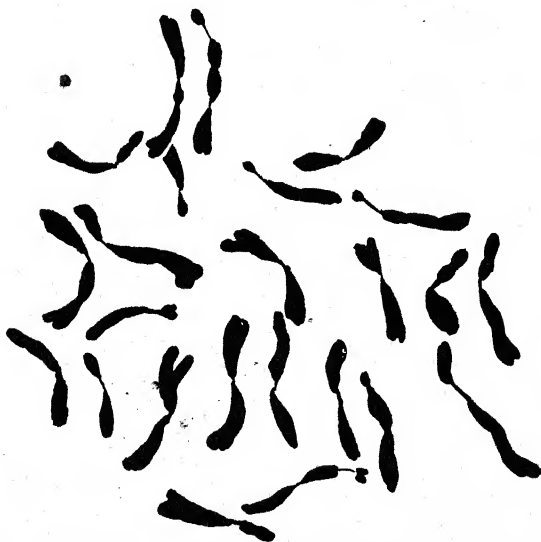
Text-fig. 5. Somatic mitosis in *Datura cornucopiae*, 2n = 24. × 6400.

The diploid-tetraploid cross (of Renner) had 22 chromosomes—the result no doubt of the functioning of a non-disjunctional gamete (cf. Text-fig. 21).

Schwemmle (1927) has stated that the chromosomes of *Oe. Berteriana* are smaller than those of *Oe. muricata*. This is not apparent from my observations of somatic divisions. But it will be noticed that the largest chromosomes in each complement are twice the size of the smallest (at meiosis as well as mitosis). The size distinctions in the hybrid observed by Schwemmle at meiosis may therefore as well be intra-specific as inter-specific. As in other cases (cf. Darlington, 1929 *d*, on *Ribes*) the difference between the species is perhaps a difference in reaction to fixatives—in which respect the anthers are more variable than the root tips.

V. MEIOSIS.

(1) NOTE ON PROPHASE.

The early prophase stages in my preparations resemble those illustrated uniformly by other workers. They are not suitable for interpretation for the general reasons given in Appendix III, but it is probable that the stages (figured in Gates, 1928) can be related with those in other organisms that have an intelligible succession (cf. Bĕlař 1928, Fig. 136) in the following way (cf. Schwemmle, 1926):

Oenothera	Other organisms
No succession of related structures	Structural succession
Synapsis or first contraction (complete collapse, Text-fig. 2)	Zygotene
Open or hollow spireme (slight collapse, Text-fig. 3)	Pachytene
"Pachynema" (Text-fig. 4) Second contraction (almost complete collapse, Text-fig. 5)[1]	Diplotene

Evidently the two stages most susceptible to change in fixation in *Oenothera*, as in most plants and animals, are zygotene and diplotene,[1] while the paired thread of pachytene is, not unnaturally, better able to withstand the effects of treatment. The fact that even the pachytene thread is partly collapsed in ring-forming *Oenothera* is, on my hypothesis (cf. Sections VII, IX and XII), due to its being single (unpaired) in the middle part of the chromosome.

(2) USUAL BEHAVIOUR.

Three of the four forms in which meiosis was studied are of the type which normally has rings of 14 chromosomes united end to end at

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[1] Gates and Sheffield (1929) seem to hold a different view: "The *raison d'être* of the second contraction is thus probably to bring the ends of appropriate chromosomes into close proximity with one another, so that the spireme may break in two places, then fuse again differently, and so cut off a ring." They, however, are seeking to discover the *purpose* of the second contraction, while I am only concerned with finding the *cause*.

diakinesis and at first metaphase (Text-fig. 6). The fourth (*Oe. biennis*, B) had rings of six and eight like Cleland's race (1925).

At diakinesis, as at metaphase, the paired chromosomes are separated by the presumably autonomous repulsion of their spindle-attachments. The connections between the chromosomes at this stage have always been represented as single, a characteristic in accordance with the theory of telosynapsis (Gates, 1930). I find on the other hand that the chromosomes are double at this stage (consisting of two chromatids), and the connections between them are double; they are connections between chromatids. This is clear when the chromatids are seen from the side (Text-fig. 6 *et al.*), but not of course when they are in the same line of
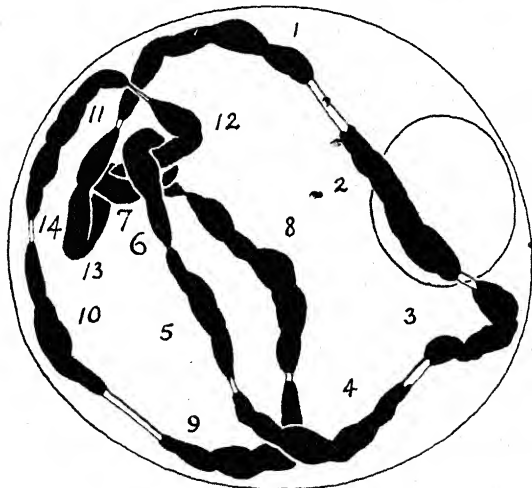
Text-fig. 6. Diakinesis in *Oe. biennis*, A. Ring of 14 chromosomes. Note double connections of terminal chiasmata seen from the side. In the longer connection only the doubleness of the ends can be seen.

vision. The interpretation is vindicated by Catcheside's observations of the triple chiasma in *Oenothera* (cf. Section VII). The association is therefore legitimately described as a "terminal chiasma," the result of the moving of the original chiasma or chiasmata away from the spindle attachment, by the process that I have called "terminalisation" to give the minimum distal association, i.e. two points. The verification of this hypothesis, so far as *Oenothera* is concerned, I shall describe later (cf. Section VI).

The rings of chromosomes are subject to the abnormalities of arrangement at metaphase and segregation at anaphase already described in

Fig. 7.
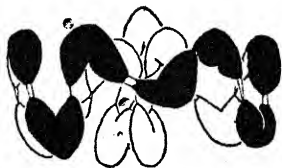
Fig. 8.

Fig. 9.
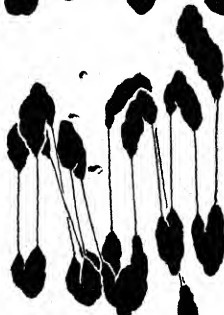
Fig. 10.

Text-figs. 7–10. Side views of metaphase and early anaphase of the first pollen mother-cell division. All chromosomes associated by terminal chiasmata. Connections are not visible in the stages shown in figs. 8–12 but are inferred from the relative position of the arms of the chromosomes. Double connections are shown where the ends of the chromosomes are seen to be double. × 6400.

Fig. 7. Ring of 14 with disjunction of all paired chromosomes; 14 chiasmata (*Oe. muricata*).

Fig. 8. Chromosomes associated by 8 chiasmata to give 6 groups thus: 1 + 1 + 2 + 3 + 3 + 4 (*Oe. biennis*, A).

Fig. 9. Chromosomes associated by 13 chiasmata to give a chain of 14 (*Oe. biennis*, C).

Fig. 10. Chromosomes associated by 12 chiasmata to give two chains of 6 and 8, which are drawn separately (*Oe. biennis*, C).

such forms by Gates, Cleland, Håkansson, Schwemmle, Kulkarni and others in *Oenothera*, and by myself in *Rhoec discolor* (1929 *c*), viz:

(i) Failure of unions between any pair of chromosomes, so that the ring may be replaced by a chain or several chains. Owing, I believe, to my sections being much thicker, I have found a much higher proportion of such breaks than other workers. In plant B for example I have found as many as five chains or separate chromosomes (Text-figs. 8 and 15).
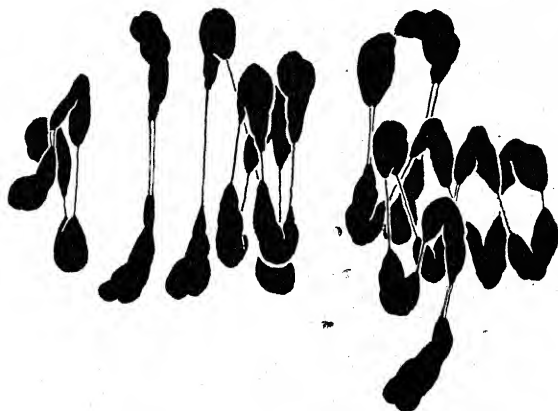
Fig. 11. Fig. 12.

Text-figs. 11 and 12. Side views of first metaphase. ×6400.

Fig. 11. Chromosomes associated by 11 chiasmata to give three chains of 2, 4 and 8 drawn separately (*Oe. biennis*, B).

Fig. 12. Chromosomes associated by 13 chiasmata to give a chain of 14 with non-disjunction, but 7 chromosomes going to each pole (*i.e.* effectively, double non-disjunction on opposite sides (*Oe. biennis*, A)).

(ii) Double non-disjunction (on the same or opposite sides) and the lagging of one of the chromosomes which have apparently failed to establish a relationship with either pole (Text-figs. 12 and 20 A). Single lagging chromosomes divide[1] as usual after the separation of the paired

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[1] I refer to chromosomes as paired when they are associated by chiasmata either terminally or interstitially. The fact that segments of one chromosome establish chiasmata with segments of two others (in a polyploid or in a structural hybrid, terminally or interstitially) does not, in my opinion, demand a special theory of meiosis to explain it. A "pair" in *Oenothera* is a ring or chain of two. A ring or chain of four is two interchanged pairs. According to the theory of telosynapsis on the other hand (Gates 1928, Cleland 1928) it does require a special theory: at one end the chromosome is associated because it is homologous with its partner, at the other end because (or although) it is not homologous with its partner. Since these two kinds of phenomena cannot be distinguished, all the chromosomes are said to be "unpaired," a description which is unnecessary and rather misleading.

chromosomes.[1] Second division plates with unequal numbers of chromosomes are observed (Text-fig. 21).

(iii) Failure of separation of the two bodies of chromosomes giving single, unreduced, second division plates (Text-fig. 21). This has been described by Schwemmle (1927) in the cross *Oe. berteriana* × *Oe. muricata.*

The first type of abnormality deserves special consideration. In all four individuals studied, complete rings are a potential maximum of association. They are formed, according to my theory, when each of the two end segments of each chromosome forms a chiasma with its homologue. When a chiasma fails to be formed between any one pair of segments there is a break in the ring, which becomes a chain. As in *Rhoeo discolor*, one or more chains may be formed as a result of failure of chiasmata between different segments, and this failure appears to be at random (as it appeared to be in *Rhoeo* where all possible combinations of chains were found), since a great variety of groupings is observed. But one cannot recognise individual chromosomes, and a statistical test of randomness is necessary. The frequencies of different numbers of chains (*i.e.* the different numbers of breaks in the potential ring) indicate that the occurrence of one break does not seriously affect the chance of the occurrence of another break, just as the failure of a chiasma between two chromosomes does not affect the chance of failure between another two. The observations which follow are recorded to show the method that must be used to ascertain the conditions that determine them. They are much fewer than those of Cleland and Kulkarni (see Section VIII), but differ in the higher proportion observed with breaks. Moreover Cleland has not distinguished between the first class and the second, while Kulkarni has only distinguished between the first class and the second. In the first case there

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[1] Their mode of division however is sometimes anomalous. Instead of the easy pulling apart of the two half-chromosomes by their attachments, as almost universally observed elsewhere, the two chromatids are seen to be in a state of tension between the attachments and the point of separation (Text-fig. 20 E). This might be attributed to the middle region of the chromosome not being paired in complex heterozygotes, so that it suffers a precocious condensation analogous to that found in the unpaired sex and *m*-chromosomes of Orthoptera and Hemiptera. If such a condensation (meaning the assumption of a spiral form by the chromosome thread) anticipated the division of the thread, anaphase splitting would meet with some resistance, and it may be surmised that such a condition prevents the splitting of the sex chromosome at the first division in certain Orthoptera. Alternatively the drawing out of the chromosome might be attributed to a weakness of the *Oenothera* chromosomes corresponding to their numerous constrictions. Similar observations have been made by Erlanson in hybrid *Rosa* (1929), and Newton and Darlington in triploid *Tulipa* (1929).

were sixteen breaks in twelve nuclei, a mean of 4/3 breaks per nucleus. If breaks at all possible points (fourteen in each nucleus) are equally

	Ring	1 chain	2 chains	3 chains	4 chains	5 chains
No. of breaks	0	1	2	3	4	5
Required no. of chiasmata	14	13	12	11	10	9
Oe. muricata						
Observed	4	3	3	1	1	0
Calculated	2·96	4·36	2·98	1·26	0·36	0·07
Oe. biennis, C.						
Observed	5	3	2	0	0	0
Calculated	4·87	3·59	1·23	0·26	0·04	—

likely, the common probability, p, is 2/21. The probability of finding n breaks in a nucleus is therefore

$$\frac{\underline{|14}}{\underline{|n}\ \underline{|14-n}}\left(\frac{19}{21}\right)^{14-n}\left(\frac{2}{21}\right)^{n}.$$

The numbers given above are calculated on this basis. In the second case there were seven breaks in ten nuclei, hence $p = 1/20$. The probability of n breaks is calculated in the same way. The measure of divergence, χ^2, between observation and calculation is 1·98 and 0·88 in the two cases. In both cases the probability of so large a divergence is greater than 0·5. Hence the agreement between theory and observation is very good. It would not be so good if breakage were much more likely to occur at one spot than at others. Expressing this result in a different way we may say that the observations agree with the assumption of a uniform metaphase chiasma frequency per pair of segments of 19/21, or 0·90, in the *Oenothera muricata* examined, and of 19/20, or 0·95, in the *Oe. biennis*.

These results therefore support my view, that the failure of union at any point in the potential ring is a failure to establish a chiasma because they can be predicted on no other view. The failure is in every way analogous to the now well-known appearance of a rod, instead of a ring, in an organism with simple pairing (such as *Primula sinensis*, Darlington, 1931 a; *Oenothera deserens*, Håkansson, 1930 a; *Matthiola*, Philp and Huskins, 1931; *Rosa*, Erlanson, 1931; *Campanula*, Gairdner and Darlington, 1930).

(3) EXCEPTIONAL BEHAVIOUR.

In a proportion of nuclei (probably 2 or 3 per cent.) of all the three forms of *Oe. biennis* there occur unions of chromosomes by interstitial chiasmata.

Interstitial chiasmata have never been described in *Oenothera*, but the configuration produced has been illustrated and discussed. Gates and

Fig 13.
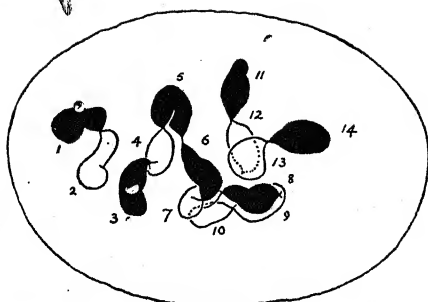
Fig. 14.
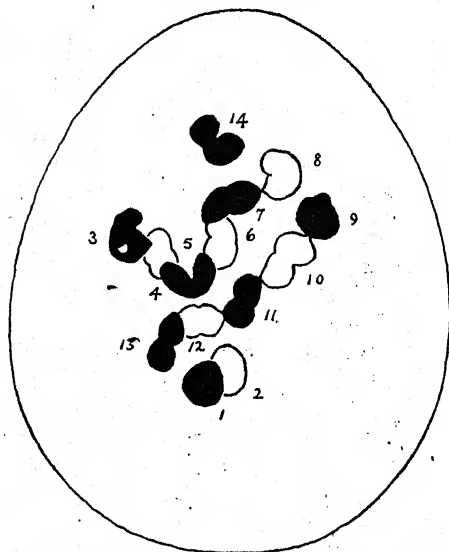
Text-figs. 13 and 14. Polar views of first metaphase (*Oe. biennis*, B). ×4800.
Fig. 13. Chains of 2, 4 and 8 chromosomes; two disjunctions on the same side.
Fig. 14. Groups of 1, 2, 5 and 6 chromosomes. No apparent non-disjunction.

Text-fig. 15. Side view of metaphase: associations of 2, 4 and 8 chromosomes. One nearly
terminal interstitial chiasma. The pair marked with an arrow has been drawn
separately (*Oe. biennis*, B). ×9400.

Thomas (1914, Fig. 37 A, reproduced by Gates, 1928) have illustrated
three pairs of chromosomes with interstitial chiasmata in the same cell.

Fig. 16.

Fig. 16 A.

Fig. 17.
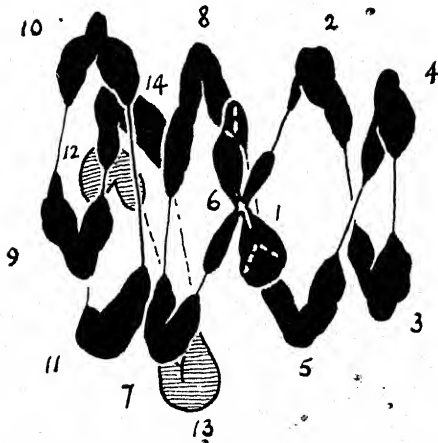
Fig. 17 A.

Text-figs. 16 and 17. Side views of metaphase in *Oe. biennis,* A and B respectively.
×9600.

Fig. 16. Chain of 14 chromosomes with 13 chiasmata, one of them interstitial. Fig. 16 A,
  separate drawing of pair with interstitial chiasma, from another cell. Note that the
  distal arms lie *at right angles to the axis of the spindle* and cf. microphotograph,
  Plate XII, figs. 9 and 10.
Fig. 17. Ring of 6 with 6 terminal chiasmata, and branched ring of 8 with 8 terminal
  chiasmata and one interstitial one. Fig. 17 A, separate drawing of a pair with
  interstitial chiasma. Note that the distal arms lie *in the axis of the spindle* and
  cf. microphotograph, Plate XII, figs. 12 to 16.

Two are unseparated and not terminally associated with other chromo-
somes (cf. my Text-fig. 12). A third is separating (cf. my Text-fig. 16)
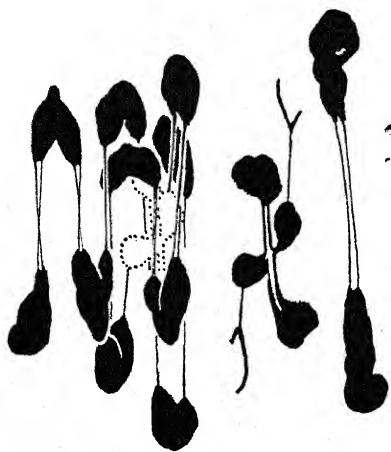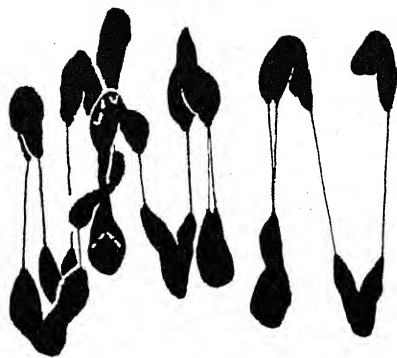
Fig. 18.   Fig. 19.

Text-figs. 18 and 19. Side views of metaphase showing interstitial chiasmata as in
Text-figs. 16 and 17 (*Oe. biennis*, C). × 6400.

Fig. 18. Chains of 2 and 12 chromosomes. The pair with an interstitial chiasma is drawn
separately to show its four (normal) terminal chiasmata.

Fig. 19. Chains of 4 and 10 chromosomes (drawn separately).

A   B   C   D   E
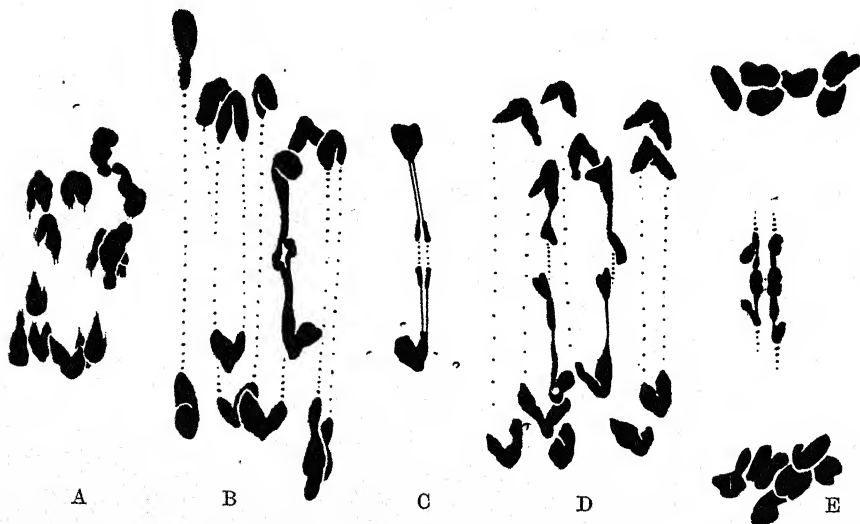
Text-fig. 20. Side views of anaphase of the first division showing chromosomes lagging for
different reasons. A. *Oe. muricata*, non-disjunctional separation with one of the
chromosomes about to be left on the equator as in *Rhoeo discolor*. B. Disjunctional
separation of several chains in *Oe. biennis*, C; one pair is lagging owing to its association
by an interstitial chiasma in the way shown in Text-fig. 16. C. Single bivalent with inter-
stitial chiasma at a later stage. D. Same stage in separation of two interstitial chias-
mata in *Oe. biennis*, A. E. Lagging univalents in same plant, exceptionally showing
tension between points of spindle attachment and point of contact of two chromatids.
× 4800.

and its appearance suggests to the authors that "some of the chromosomes are losing some of their chromatin, a trail of which is left on the spindle as the chromosomes pass to the poles." This appearance is due to the tension between the attachment constriction and the separating chromatids, which is characteristic of the separation of interstitial chiasmata and is still frequently misinterpreted. It has been shown by Bělař in the living cell (1928 *et al.*). The nucleus is cut (sections are 10 μ thick) and the cross-arms are apparently interpreted as extra whole chromosomes.

Håkansson has also illustrated such structures in trisomic forms (1930, 1 *b* and *d*, 2 *c*, 4 *e*, 5 *b* and *f*) and described the segments distal to the chiasma as a cross-arm ("*Querarm*"). He has suggested the analogy with chiasmata observed in other organisms.
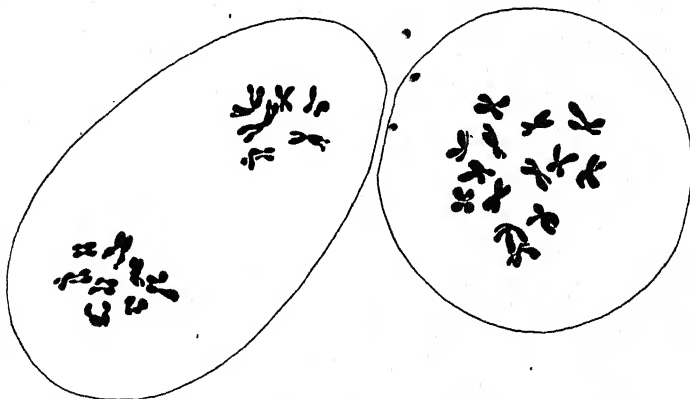
Text-fig. 21. Metaphase of the second division in *Oe. muricata*. Left, following non-disjunctional (6 + 8). Right, following formation of restitution nucleus such as will give unreduced gametes (*n* = 14). ×3600.

Similarly in hybrids of *Datura*, which are analogous with *Oenothera* in that they give evidence of interchange and normally have complete terminalisation, "humps" have been referred to (Bergner and Blakeslee, 1930) which are probably the distal arms of interstitial chiasmata. If they are constant, they are, as I shall show, of significance in the study of variation in the arrangement of the chromatin material (cf. Erlanson, 1931).

In these diploid forms three types of structure are observed at metaphase in consequence of the maintenance of the interstitial chiasma. The first (Text-fig. 15) is that in which the arms distal to the chiasma are so short that they merely lead to a thickening of the double connection

between the pairing chromosomes, which are held closer together than usual. In the second (Text-fig. 16 and Pl. XII, figs. 9–10) the distal arms are longer, the whole configuration is of a striking cross-shape and the exchange of partners amongst the four chromatids can be seen. At anaphase (Text-fig. 20 B and microphotograph Pl. XII, figs. 17–19) the chromosomes with the interstitial chiasma lag behind those with the terminal chiasmata, as I pointed out would be the case (Newton and Darlington, 1930, p. 12). As the chromosomes move apart the structure of the chiasma and the existence of the four chromatids can again be seen (Text-figs. 20 B and C). Such lagging of two chromosomes symmetrically associated by *terminal* chiasmata is never observed in *Oenothera* or any other organism. It is characteristic of the *interstitial* chiasma.

The third type of structure observed is more complex. Here the distal or cross-arms are associated by terminal chiasmata with two other chromosomes (Text-fig. 17 and Pl. XII, figs. 11–16). In this type the chromosomes between which the interstitial chiasma occurs are therefore not those normally associated terminally in the ring. The complete association if spread out would appear as a "figure-of-eight."

Interstitial chiasmata (as opposed to subterminal chiasmata which are the result of occasional failure of complete terminalisation as in the first type) have been observed nine times in side views of the first metaphase and four times at anaphase. In one of the metaphase observations, one of the distal segments is terminally associated; in four of them, both are terminally associated with other chromosomes.

The theoretical implications of the figure-of-eight pairing are several. Before considering them I would therefore point out the basis of its interpretation. It depends, not only on the connections observed between the chromosomes, but also on the positions and attitudes of the chromosomes which seem to permit of no other interpretation. Where the arms are, as in the second type, unconnected distally, they lie at right angles to the spindle. In the figure-of-eight, on the other hand, as shown by the microphotographs, the two arms are turned in the axis of the spindle, and towards the chromosomes with which they are terminally connected. The contrast is best seen by comparing Text-figs. 16 and 17.

In Text-fig. 28 a figure-of-eight configuration is illustrated diagrammatically. In this the interstitial chiasma is shown between chromosomes separated by four chromosomes on one side and six on the other (in a ring of twelve). In the plants studied it is important to notice that the number of chromosomes intercalated between the two forming the interstitial chiasma is also an even number in every case. In plant C

(Text-figs. 18 and 19) there are four chromosomes on one side and eight on the other (in a ring of fourteen). In plant A (Text-fig. 17) there are two chromosomes on one side, four on the other (in a ring of eight). In plant B the position was evidently the same (although the relations of all the chromosomes could not be determined), for this must always be so where the separation of all the chromosomes, including those associated interstitially, is disjunctional. Other configurations, however, were observed in which the chromosomes, apparently associated interstitially, are passing to the same pole ("non-disjunctionally"). This association is necessarily more difficult to interpret, and has not been illustrated. The type illustrated is that referred to as potentially responsible for the origin of half-mutants in multiple-ring forms; the type not illustrated is referred to as potentially responsible for the origin of multiple rings from simple pairs and of mass-mutants from multiple-ring forms (Section X).

VI. SIGNIFICANCE OF THE "FIGURE-OF-EIGHT".

The figure-of-eight configuration affords a demonstration of the following five principles of special or general interest:

(i) Interstitial chiasmata are characteristic of parasynapsis, and have never been shown to arise except from side-by-side conjugation of chromosomes. *The observations demonstrate parasynapsis in* Oenothera *because they were predicted on the assumption of parasynapsis*[1] (and contrary to the assumption of telosynapsis).

(ii) When an end segment of a chromosome *A* is homologous with an end segment of a chromosome *B*, their middle segments need not be homologous. The middle regions of the chromosomes of ring-forming types of *Oenothera* do not therefore continue the homologies of the distal segments. A pair is associated interstitially different from any that are ever associated terminally. This is therefore *cytological evidence that the ring*

[1] The occurrence of the structures I have described can be predicted from four statements of mine following from the theory of parasynapsis: (i) "occurrence of interchange between non-corresponding segments of opposite complexes [of *Oenothera Lamarckiana*]... will give a new type with a ring of six and four pairs" (1929 *a*); (ii) "it is possible to regard segmental interchange between non-homologous chromosomes as the result of crossing-over between small, relatively translocated segments" (1930 *a*); (iii) "terminalisation from chiasmata formed in such segments would therefore be impossible" (1929 *c*); and (iv) "where a chiasma is formed amongst four chromatids an interchange of segments (genetic crossing-over) has occurred between them" (1930 *a*). These statements constitute a prediction that interstitial chiasmata (giving a figure-of-eight) would be found to occur and be preserved at metaphase between chromosomes not capable of terminal association in the particular ring-forming *Oenothera* individual. I recall them in order to show that the theory of parasynapsis and terminalisation in *Oenothera* is a verifiable working hypothesis.

forms are complex heterozygotes: *the basis of the heterozygosis is translocation.*
The mid-regions of the chromosomes must be the seat of the Renner
complex. This will be discussed later (Section VIII). Meanwhile I shall
refer to the chromosomes of ring-forming individuals by the capital letters
A–P and to the middle regions by the small initials of the complex in
question, together with such other letters as occasional chiasma forma-
tion may indicate, *e.g. x* in the present case for the homologous interstitial
regions which form occasional chiasmata (see Section X).

(iii) I have suggested two possible grounds for failure of termina-
lisation in *Tradescantia* (1929 *c*, 1931 *b*, cf. footnote p. 421), viz. (1) incom-
plete movement conditioned by genetical or environmental factors and
(2) arrest of movement due to a change of homology. The first type of
interstitial chiasma is probably due to the first condition; the second,
sometimes so; but, since the third appears under my predicted conditions
(change of homology), I consider that these conditions must be held
responsible. Here it follows from the observed pairing that a change
of homology takes place between the chiasma and the ends of the chro-
mosomes. Here therefore is *evidence of the arrest of terminalisation owing
to change of homology* as expected in structural hybrids and suggested
(but not proved) by observations of *Tradescantia*. In such cases a strain
must be imposed equally on the four chromatids at the point and at
the·time at which the movement is arrested. Such a strain would not
occur in terminalisation where there is no structural obstacle. Since
breakage does not occur in these exceptional circumstances it is difficult
to suppose that it could take place in normal circumstances in a way in
which (as I once imagined, 1929 *b*, p. 52) it might lead to crossing-over
(cf. 1931 *c*).

(iv) On purely cytological assumptions these configurations are
*evidence that "genetic" crossing-over has taken place at the interstitial
chiasma* in every case. Moreover this demonstration is of an entirely
different character from that provided by the quadrivalent configurations
in *Hyacinthus* (see Appendix IV), as follows.

The individuals under investigation are disomic and not tetrasomic,
for they have none of the configurations that are possible in tetrasomic
organisms with terminal association (cf. Darlington, 1931 *a* and *b*).

Now in the diagram of metaphase, Text-fig. 22, the segments *A* of the
chromatids *AB* and *AF* are homologous with the segments *A* of the
chromatids *AN*, *AN* (since pairing occurs only between homologous
segments). Similarly the segments *G* of the chromatids *GB* and *GF* are
homologous with the segments *G* of the chromatids *GH*. But, in the

absence of crossing-over in the segment x, the A segment of AB being a continuation of the same chromatid as the G segment of the GB chromatid, A must be homologous with G and the organism tetrasomic in respect of this segment. Which is absurd, as shown above.
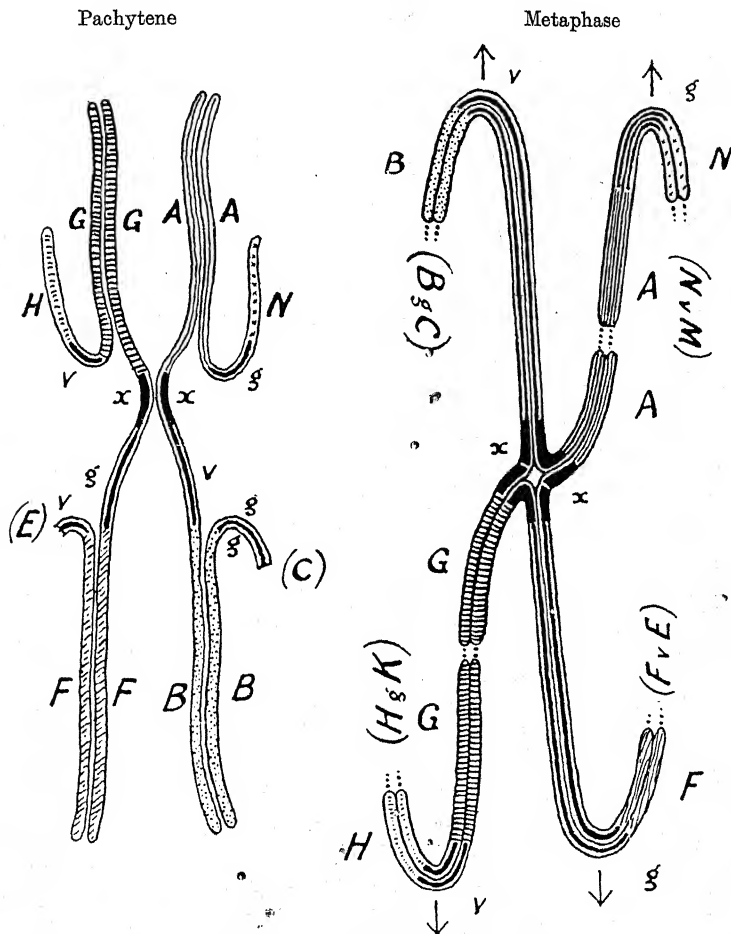
Text-fig. 22. Diagram showing pachytene association (of chromosomes) and metaphase pairing (of chromatids) of the chromosomes concerned in the branched ring (figure-of-eight). The formulae are taken from the hypothesis of the origin of half-mutants in *Lamarckiana*. The chiasma in the x region is represented for simplicity as not having suffered terminalisation. The point of crossing-over is marked by a gap in the two chromatids. Adjoining chromosomes represented by formulae in parentheses. The *velans* and *gaudens* complex differential segments of *Lamarckiana* are marked v and g.

Therefore crossing-over must take place between two of the four chromatids (one with an A segment and one with a G) in the segment x

and be correlated with the formation of the chiasma. The implications of this view have been considered elsewhere (1930 *b*, 1931 *a* and *b*).

(v) Since crossing-over occurs interstitially between chromosomes which are distally non-homologous, this is *an observation of the mechanism which will lead to the "interchange of non-homologous segments"* or *"seg-mental interchange between two non-homologous chromosomes" in-ferred* by Belling and Blakeslee in *Datura* (1924, 1926) and by Muller (1930 *a* and *b*) in *Drosophila*.

The importance of this in regard to mutation will be considered later (Section X).

VII. THE METHOD OF CHROMOSOME PAIRING IN *OENOTHERA*.

The prophase of meiosis in *Oenothera* has hitherto, for the reason stated above (Section V (1)), proved unintelligible. The evidence of obser-vation offered for telosynapsis by Gates (1928 *et al.*) and Cleland (1929 *et al.*) and for parasynapsis by Boedijn (1924) and others is not such as to bring conviction to those accustomed to more favourable material (see Appendix III). Both kinds of evidence depend on misinterpretation of the values, in terms of somatic chromosomes, of the bodies observed at the different stages of prophase.

The evidence from analogy (Schwemmle, 1926 and Kihara, 1927) that *Oenothera* may be considered parasynaptic has been shown to be valid by later cytological work, including the present study. Schwemmle and Kihara did not, however, make the necessary genetical assumptions: these were made later by Håkansson and myself.

It has been shown in four organisms how a prophase with parasy-napsis can give diakinesis and metaphase of the kind found in *Oenothera* (*Campanula persicifolia*, Gairdner and Darlington, 1930; *Primula sinen-sis*, Darlington, 1931 *a*; *Rosa*, Erlanson, 1931; *Matthiola*, Philp and Huskins, 1931). It has also been shown that multiple chiasmata are formed in polyploid *Oenothera* (Catcheside, 1931), as I had shown them in polyploid *Tradescantia* following parasynapsis (1929 *b*). In these the separate connections of the two chromatids of each chromosome can be seen. The reason for this is that, while the two connections in a simple chiasma are close, and therefore easily collapsed by fixation, in a triple chiasma the three connections form a triangle and are therefore not so liable to collapse. Gates' conclusion (1930) that the connections between pairing chromosomes in *Oenothera* "are invariably single" (as apparently required by the hypothesis of telosynapsis) is therefore unsound.

There is thus every cytological reason to suppose that the assumption

of parasynapsis is valid, and that meiosis follows the general course of type C (Darlington, 1931 *b*), described hypothetically in *Tradescantia*, and from observation in *Campanula, Primula, Rosa, Matthiola* and other genera, as follows:

(1) Homologous parts of chromosomes pair side by side.

(2) Chiasmata are formed at random amongst the paired elements, so that most pairs of segments (*i.e.* parts of chromosomes between the attachment constriction and one end) form one or more chiasmata. The mean number at diplotene is probably three per paired chromosome.

(3) Chiasmata are terminalised, *i.e.* they move towards the ends of the chromosomes so that most ends of chromosomes are associated (with homologous ends of other chromosomes).

(4) All association at diakinesis and metaphase is normally by terminal chiasmata. Their frequency is reduced by terminalisation to about 1·9 per chromosome (cf. Section V (2)).

(5) Since terminalisation is probably away from the attachment constriction, this point must not lie within one of the interchanged segments. For, if it does, chiasmata will be formed on either side of it and give metaphase configurations in the ring with two chromosomes associated in both arms, one interstitially and the other terminally, as shown in *Rosa* (with irregular structural hybridity), but not in *Oenothera*.

(6) "Breaks" in the ring of chromosomes are due to the failure of pairs of segments to establish chiasmata.

We must therefore compare the frequencies of different numbers of breaks with chiasma frequencies in other plants.

Cleland (1929) and Kulkarni (1929 *b*) have recorded observations of break-frequency, but both series of observations are incomplete. Calculating from Cleland's observation of 3 per cent. of nuclei with two breaks in *Oe. Lamarckiana*, the frequency with one break should be 25 per cent. on a random expectation. This agrees closely with Kulkarni's observations in *Oe. pratincola* of 31 per cent. at diakinesis, and 29 per cent. at metaphase of nuclei with one break (presumably including those with more than one break). Both these observations and my own agree with the assumption of randomness required by the view that metaphase association is determined by chiasma formation as in other plants. Cleland's and Kulkarni's observations both agree with the assumption that the chance of a break occurring is a little over 0·02, or a metaphase chiasma frequency of a little under 0·98 per segment pair. My own agree with a chiasma frequency of 0·90 per segment pair in one form and 0·95 in another (see Section VIII (1)).

The change between pachytene and diakinesis in a ring of four chromosomes and its structure in relation to crossing-over may be represented as in the diagram (Text-fig. 23, cf. diagram, 1929 *a*, p. 348).

Such a system of pairing will give the following types of association at diakinesis and metaphase in a ring-forming *Oenothera*—(i) to (v) in diploids:

(i) A certain potential ring or rings (including ring pairs) of $2n$ chromosomes associated by $2n$ terminal chiasmata.

(ii) A chain or chains of chromosomes associated by fewer chiasmata.
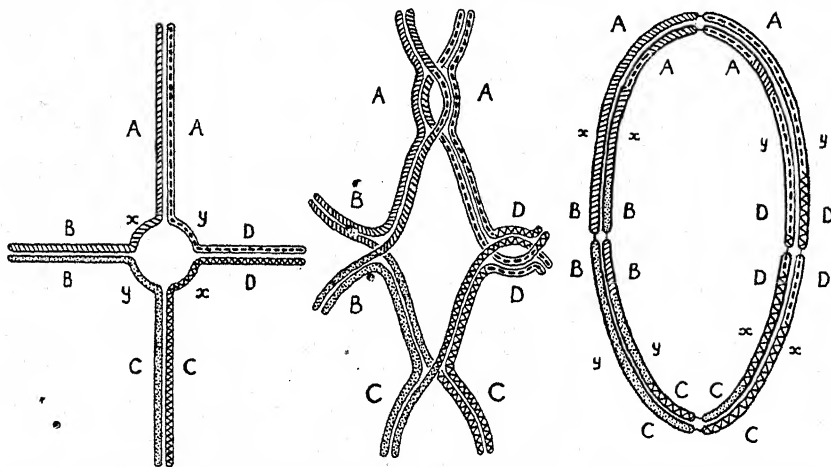
Text-fig. 23. Diagram to show how crossing-over will occur in a diploid ring of 4 chromosomes. The three stages represented are (i) pachytene, (ii) diplotene, (iii) diakinesis (after terminalisation of chiasmata). The chromatids are represented separately in the second and third. The chromosomes are illustrated to distinguish them as units in order to show the effect on their constitution of crossing-over (single and double, progressive and recurrent) and not according to the homologies of their segments. The middle segments of one complex are *x, x* and of the other *y, y* (Section IX). The scheme is applicable, *mutatis mutandis*, to chromatid arrangement and crossing-over amongst 4 chromatids in a homozygous tetraploid with terminalisation (such as *Primula sinensis*) when the behaviour illustrated is such as might give double reduction (Darlington 1929 *c*, 1931 *a*; Haldane, 1930).

(iii) Interlocking of rings as a result of one chromosome passing between two others at pachytene in the neighbourhood of the attachment constriction, with chiasmata formed on either side of the interlocking at diplotene, and their terminalisation in *opposite* directions.

(iv) Interlocking of rings (as shown in the diagram, Text-fig. 24) as a result of one chromosome passing between two others at pachytene in a distal region, with chiasmata formed on either side of the interlocking, and terminalising in the *same* direction. The configuration produced will resemble that illustrated by Cleland (1925, 1926 *a*) in *Oe. biennis*. I do not

agree with Gates (1930), that the details of these fine structures will be clearly visible in all circumstances in *Oenothera* because they are visible in *Hyacinthus* and *Tradescantia*, with chromosomes one hundred times as big; for accuracy of observation in cytology is, in my opinion, a function of the size of the object observed.

(v) Interstitial chiasmata will be formed where segments of chromosome, whose homology does not continue to the ends of the chromosome, associate at pachytene and form chiasmata at diplotene.

(vi) In triploids and tetraploids triple and quadruple chiasmata will be formed (Darlington, 1929 *c*; Catcheside, 1931). In triploids anomalous interstitial chiasmata will be formed more frequently, other things being equal, owing to the third unpaired chromosome having greater opportunity
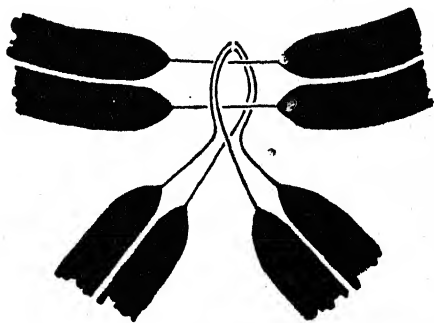
Text-fig. 24. Diagram to show the effect at metaphase of interlocking at pachytene between two chiasmata terminalising to the same end of the chromosome. Result: fused rings.

for associating in parts that do not follow the homology of the normally pairing segments (cf. Håkansson, 1930 *b*).

The same principle applies to the haploid, where reduplication of an interstitial segment within the haploid set will lead to the occasional formation of an interstitial chiasma, although in the normal diploid (the parent of a haploid *Oenothera* has always been homozygous and if heterozygous it must always be capable of throwing homozygous mutants) such segments would be paired in accordance with the homologies of the segments on either side of them. This probably accounts for the exceptional pairing noted in a haploid (cf. Appendix V) (Emerson, 1929).

These variations cover all the forms of chromosome association observed in *Oenothera*.[1]

[1] The observation of variation in the size of the ring in *Oe. Agari* (Sheffield, 1929) is not convincing, for it involves another unparalleled observation, viz. odd-numbered rings in a diploid, and is illustrated from cut nuclei.

VIII. STRUCTURAL HYBRIDITY AND THE RENNER COMPLEX.

(1) APPARENTLY CONTRADICTORY PROPERTIES OF THE RING-FORMING CHROMOSOMES.

We are justified in concluding from the behaviour of the complex-heterozygote *Oenothera* species that the end segments of their chromosomes have a special relationship which we can determine on the assumption that pairing takes place only between likes. How much does this tell us? In a homozygous form we can represent a pair of chromosomes as made up of identical segments throughout, *e.g. abcdefg* and *abcdefg*. But are we justified in concluding that the chromosomes in a ring-forming *heterozygote* are each made up of two segments, each of which corresponds wholly with a segment in another chromosome? Evidently we are not, for the following reasons:

(i) *The set of chromosomes of one complex is distinguished from that of the other complex by a sharp genetical difference or group of differences. Crossing-over does not take place in regard to these differences.* They constitute the "lethal factors" of Müller (1917, 1918) and the "complex" of Renner (1917, 1925 *et al.*). They occur in every gradation of intensity, *e.g. (a)* those which absolutely inhibit the occurrence of individuals homozygous for even a part of the complex, as in the *albicans-rubens* combination (*Oe. biennis* (8) + (6)),[1] which do not segregate homozygotes from either independent part of the complex; (*b*) those which segregate crippled plants, presumably homozygotes, from part of the complex as in *truncans-gaudens* ((10) + (4), Cleland and Oehlkers, 1929); (*c*) those which segregate normal homozygotes from part of the complex as in *velans-gaudens* (giving half-mutants *subvelans* and *paenevelans*, or the mass-mutant of *Oe. pratincola, vide* Section X) or *flavens-subcurvans* (Rudloff, 1930; (6) + 2 (4)); (*d*) those which segregate normal homozygotes from the whole complex, changed only by crossing-over with an exceptional partner, as in *flavens-rubens* (from *suaveolens* × *biennis*) giving *flavens-flavens* or "*lutescens*" (Renner, 1927) which has seven pairs (Hoeppener and Renner, 1929).

(ii) *In spite of the hybridity of the ring, pairing is extraordinarily regular.* Many homozygous plants which normally form bivalent rings (*Primula, Matthiola, Oe. deserens,* Håkansson, 1930 *a*, Fig. 19) also produce, by failure of the chiasma in one arm of the paired chromosomes, a rod united by a single chiasma. Yet the proportion of failure of chiasmata is apparently very small in the heterozygous ring forms of *Oenothera*.

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[1] See Abbreviations, Section XIII and Configuration List, Appendix II.

The chiasma frequency in all forms is definitely greater than 0·9 per pair of segments, which is comparable with that in homozygous diploid *Primula* (Darlington, 1931 *a*, 1·91 per pair of chromosomes), *Matthiola* (Philp and Huskins, 1931), and in *Rosa* (Erlanson, 1931).

Another kind of evidence of the prophase frequency of chiasmata is afforded by Catcheside's observations (1931) of chiasmata in a triploid *Oenothera*. The frequency determined corresponds to the terminalisation of two prophase chiasmata per set of three chromosomes, the same number as in the diploid set of two. In trisomic *Datura*, Belling (1927, Table I) found associations which, on the same interpretation, require a mean number of prophase chiasmata of 2·25 per set of three chromosomes, while in the diploid 2·0 are found. The chiasma frequency in this *Oenothera* is therefore a little lower than in *Datura*, where the chiasma frequency is higher than in *Primula* (Darlington, 1931 *a*).

Apparently chiasma frequency in *Oenothera* is just sufficient to ensure fairly regular pairing of the two ends of each chromosome in the diploid, but not the association of three ends in the triploid.

We have, therefore, a group of plants with the apparently contradictory properties of (i) chiasma-formation and pairing of the chromosomes as regular as that in various related and unrelated homozygotes, (ii) a high degree of heterozygosity, and (iii) the anomalous property of no crossing-over of dissimilarities between the complexes although crossing-over does occur outside the complexes.

(2) The necessary assumption: segmental differentiation.

(i) Since metaphase pairing follows the terminalisation of chiasmata between elements which were paired and continuously homologous at pachytene (any change of homology leading to arrest of terminalisation), it follows that the pairs of distal elements of the chromosomes in all *Onagra* species are continuously homologous, *i.e.* have the same linear arrangement of particles; and any differences between pairing chromosomes are proximal to these pairing elements.

(ii) Since the distribution of chromatids at reduction is determined by their continuity with the chromatids associated at the attachment constriction, it follows that, if no crossing-over takes place between complexes, no pairing can regularly occur, and no chiasmata (with crossing-over) can be formed, in the region between the dissimilarities and the point of attachment.

These arguments, the one cytological, the other partly genetical, lead to the same conclusion, viz. the differences between complexes in ring-

forming *Oenothera* (Renner's "Rest") are concentrated in the middle regions. *Each chromosome is made up of two terminal "pairing segments" and one median "differential segment."*

Crossing-over must be restricted to the terminal regions. Such a property of restriction is only found elsewhere in organisms with dimorphic sex chromosomes, in which it seems also necessary to assume a restriction of crossing-over in the complement as a whole in the heterozygous individuals, i.e. in one sex (Darlington, 1931 *a*).

The kind of differentiation of the middle regions is indicated by my observation that the anomalous interstitial chiasmata are formed between them (Section VI (ii)). The middle regions of the chromosomes forming one complex consist of an arrangement of chromatin unrelated with the corresponding regions in the chromosomes forming the opposite complex, but probably related genetically in that the same materials are present but in different proportions. As a rule, each is probably deficient in materials over-represented in the other, so that the genetic compositions of the gametes will suit them for different conditions, while the genetic composition of the zygote will differ less markedly from that of homozygous individuals (Sections VIII (1) (ii) and X (2)). How this could come about will be considered in the next sections.

In view of the unidentified relationship of the particles in the middle segments, it is necessary, for example, to label the six differential segments of the *gaudens* chromosomes *g* and those of the *velans* chromosomes *v* in *Oe. Lamarckiana*. The assumption of the specific pairing ends (indicated by capital letters) undifferentiated from their homologues throughout the *Onagra* group is an assumption in cytological terms paralleled by Renner's genetic assumption (1927) that "ein Teil des sogenannten Keimplasmas muss doch wohl ziemlich stabile Struktur haben."

(iii) There are two ways in which such a system might arise. A genetically conditioned restriction of chiasmata to the distal parts of the chromosomes (as they are restricted to the proximal parts in *Fritillaria Meleagris*, Newton and Darlington, 1930) would separate the functions of the proximal and distal parts in the ring, for (on the hypothesis of crossing-over that I have defined, 1930 *b*) the proximal parts of the chromosomes would then vary independently of one another in the two complexes and such differences as would arise between segments would accumulate so as to prevent any pairing were it genetically permitted. Such an assumption I regard as a plausible explanation of the origin of heteromorphic sex chromosomes and of the sexual dimorphism they determine (1931 *a*).

One observation appears to be in favour of this view. Bivalent rings are seen interlocking more frequently in *Œnothera* (to judge by illustrations of Cleland, Hoeppener and Renner 1929, *t.f.* 30 and others) than in other plants with similar ring bivalents (*e.g. Matthiola, Primula*). In this respect *Œnothera* is analogous to those Orthoptera with a polarised bouquet stage (zygotene) in which pairing takes place last in the middle. It is evident that the effect on interlocking would be the same in the *absence* of pairing in the middle region as with its postponement (cf. Section VII). It is also possible that *Œnothera* has some polarisation at leptotem.

But it seems more probable that the differentiation of median segments in heterozygous *Œnothera* has proceeded directly from the random occurrence of structural changes of the kind inferred (Section VI (ii)), unassisted by restriction of chiasmata, for two reasons: (i) that segmental interchange occurring in the way I have supposed will itself follow translocation and will lead to reduplication (Section X (2) A) and (ii) that I have shown crossing-over to occur exceptionally in the "protected" regions. This shows the protection to be due to structural dissimilarity rather than to genetical restriction of pairing or chiasma-formation.

(3) CORRELATIVE EVIDENCE.

The development of such a system can be shown to be favoured by natural selection and it will operate in accordance with observation.

(i) The first advantage of the system of segmental specialisation is in the way in which the structural changes which have given rise to the complex have been perpetuated. They have been subject to natural selection of a very special kind. We see it best exemplified in *Oe. muricata* and some of its relatives.

This species consists of two complexes *curvans* and *rigens* (Renner, 1917). Each of these, first, consists of fourteen terminal pairing segments which are the common property of all the species of *Onagra*; in these, crossing-over occurs with chiasma formation, but being between likes has no genetical result. These segments are associated in pairs in a way that is characteristic of the complex. They have been perpetuated in the association in which we find them on account of their arrangement giving a ring mechanism of segregation, which is profitable to the race. The terminal segments are therefore selected only according to their arrangement and as instruments of segregation.

Each set of chromosomes consists, secondly, of seven median non-pairing "*differential segments*" which determine the genetic characters

peculiar to the set. This "complex-differential," varying independently of its partner differential (for they cannot normally mix), is subject to an exceptional kind of selection. First, its variation will be limited as a constituent of the zygote to which it will contribute a part of its characteristic distinctions from other species. Secondly, in the gamete its variation will be limited by its success either as pollen grains or megaspores, or as both. Thus, if one complex is more successful as pollen, then any greater success of the other complex in competition for embryo-sac production (Renner, 1921) will give a direct increase in fertility.

The separation of functions between the pairing segments and the differential segments of the ring chromosomes has therefore made it possible to develop an elaborate mechanical-physiological device of the *Oe. muricata* type, where two kinds of gametes are produced: one, *curvans*, effective as the pollen; the other, *rigens*, as the megaspore, so that a diploid hybrid may breed true with scarcely reduced fertility.

(ii) A second advantage of the specialisation of function in the segments seems to be that hybridisation may take place within a group of species showing considerable diversity of form without any appreciable loss of regularity in chromosome pairing. The pairing ends are uniform throughout the group.

(iii) Chromosomes in a ring have non-pairing differential segments which are protected from any relationship with corresponding segments in the chromosomes associated with their end segments. Each chromosome in a ring (no matter whether in an old "species," or in a new "hybrid") will therefore have a characteristic constitution, and the organism with the ring will produce two different types of gametes. It will be heterozygous, although true-breeding.

Chromosomes which associate in pairs at meiosis will have two kinds of relationships. In an interspecific hybrid in which the two complexes have the same pairing properties (*i.e.* give seven pairs) their middle segments will have had some chance of differentiation, although probably not so much as if their pairing ends were differently arranged, for the similarity will indicate a close common ancestry. Such a hybrid is the *flavens . stringens* combination of Oehlkers or *flavens . hpurpurata* of Rudloff (Appendix II).

On the other hand, in derivatives of hybrids (such as *flavens . subcurvans* of Rudloff, 1930), and in mutants (such as *deserens*), association in pairs usually indicates that the chromosomes so associated are identical, because two chromosomes so associated can only be derived from the same chromosome in a ring-forming parent owing to the constancy and

specificity of pairing properties. The greater commonness of this type accounts for the general rule (Oehlkers, 1926; Cleland, 1928) that ring-forming segregates of *Oenothera* crosses are hybrids, while bivalent-forming segregates are homozygous.[1]

(iv) Dissimilar complexes cannot as a rule exchange material (cross-over) because, (1) they have no continuous linear homology with one another, so that they will not (as a rule) pair to cross-over within the chromosome (cf. Appendix V), and (2) their units (the middle segments of individual chromosomes) are associated with different pairs of end segments in the opposite complexes (on my original hypothesis of specificity of pairing properties). Crossing-over will therefore give mutants which will usually be non-viable (Section X). Exceptional exchanges between complexes will occur most frequently where the differentiation of the complexes is least: hence *Oe. muricata* is more stable than *Oe. Lamarckiana*.

(v) Unrelated variation in the differential segments of complexes will lead to the very occasional formation of chiasmata between the small homologous segments. These must be only occasional because (1) the segments are short and therefore with complete association will have a chiasma frequency much below unity, and (2) the segments, not continuing the homologies of the regions on either side of them, will rarely have the opportunity of pachytene association (cf. Darlington, 1930 c). But anomalous chiasma formation, if determined by such conditions, should occur with different frequency in forms of different constitutions. For example it should occur less frequently in forms of great stability such as the *Oenothera muricata* I have examined. It should occur with greater frequency in trisomic and triploid forms for this reason: in trisomic forms there is always one particle of chromosome, of each three homologous particles, left unpaired at pachytene (Newton and Darlington, 1929). This particle will have, in the case of the unpaired differential segment, a greater opportunity of finding a homologue than in the case of a diploid where pairing is always complete on either side of it. Håkansson's

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[1] It should be noted that Gates' and Sheffield's observation of seven pairs in a derivative of a cross between two hybrids (1929) is not, as they think (pp. 386–7), at variance with this conclusion. For it is a corollary of Mendel's first law that a cross between two heterozygotes (and, *a fortiori*, its derivatives) may be homozygous. And this principle has been demonstrated most strikingly in *Oenothera* (Renner 1927). Gates' and Sheffield's "hybrid" was, like *lutescens*, a homozygous "hybrid." Nor is their observation of reciprocal differences amongst individual derivatives of crosses between complex heterozygotes evidence of cytoplasmic influence, since such differences are expected from Renner's observations of segregation in the heterogametic parental species (cf. Hoeppener and Renner, 1929, pp. 75–77).

observation (1930 *b*) that "bei den von mir studierten Trisomen waren sie jedoch mehr oder weniger häufig vorhanden, bei 14-chromosomigen Formen kommen dagegen anscheinend Querarme sehr selten oder gar nicht in den Bivalenten vor" is therefore in agreement with expectation. It is analogous to the observation that associations of higher than three are commoner in triploid *Pyrus* than associations higher than two in the diploid. The odd chromosomes are free to associate in discontinuous segments (Darlington and Moffett, 1930 and Appendix V).

(vi) Crossing-over may take place between differential *A* and differential *B*, although crossing-over involving the same factors cannot take place between differential *A* and differential *C*. This follows from the hypothesis for the differential region in complex heterozygotes must be variable, being relative to the opposite differential (*vide* note at end of section). In such a way we can understand that a homozygous *flavens* may be extracted from hybrids of *Oe. suaveolens* with other species, although it may not take place in *Oe. suaveolens* itself.

(4) RECAPITULATION.

Summing up: the arrest of an interstitial chiasma by change of homology proves that the homology of the distal ends of the chromosomes in ring-forming *Oenothera* species must be continuous and any structural changes therefore must be confined to the middle regions of the chromosomes. These regions are the *complex-differentials*.

This agrees with two sets of observations: (1) the absence of crossing-over between the complexes; because if chiasmata could be formed proximal to the differentiated regions of the complex there would be crossing-over between complexes, whereas if the dissimilarities are in a central block this cannot occur; (2) the regularity of pairing observed in hybrids between dissimilar species within the *Onagra* group, for the differences between these species are confined to the regions which do not pair in the species themselves.

The assumption of an unpaired differential region means that the "complex" as a whole is a lethal system balanced by absence of crossing-over. The arrangement of materials in the differential region constitutes its physiological character as a complex and as a lethal system, as well as its mechanical character as a unit incapable of crossing-over within the chromosome. The structural association of a particular differential segment with two end segments unlike the two with which any other differential segment is associated also prevents crossing-over between whole chromosomes.

In this way it is possible to express Muller's balanced lethals (1917, 1918) and Renner's complexes (1917, 1925)—purely genetical conceptions —in terms of the organisation of the material of the chromosomes. The arguments that I have used in favour of my cytological statement are necessarily, on the genetical side, the same as those they have used in favour of their genetical statements.

These considerations support the assumption that all differences between complexes (*i.e.* all variations) in *Oenothera* are and perhaps determined by structural change (cf. Section X (3)).

NOTE.

Confusion is possible with regard to the use of the term "segment." It has an absolute significance as applied to structural changes such as translocation and interchange. It has only a relative significance as applied to the description of the homologies of chromosomes. For example, two chromosomes *abcdef* and *abcghk* must be described relative to one another as each consisting of two segments, the one *abc* and *def*, the other *abc* and *ghk*. But two chromosomes *abcdef* and *abcgek* consist each of four segments. This is why, although it is possible to describe the constitution of seven *Oenothera* chromosomes in respect of their pairing properties in terms of fourteen segments, the description in no way represents a final analysis of the structural constitution of the complex. It is also the reason why the pairing segments of a chromosome form larger or smaller parts of it (crossing-over therefore to a greater or less degree) according to the constitution of the chromosomes opposed to it.

The variability is well exemplified by a comparison of *Oe. Lamarckiana* and its mutant *rubrinervis* according to my hypothesis (Section X). For, in the species, *x* is not part of the effective pairing segment *A*. In the mutant it is continuous with it in both complexes, and therefore forms a pairing unit. This differentiation of segments of one set of chromosomes therefore has no meaning except in relation to the opposite set.

IX. THE CROSSING-OVER MAP.

A crossing-over map may express in geometrical form the resultant of a variety of mechanical and physiological conditions. To determine what such a map should be in ring-forming plants is therefore a task of general value; for the different conditions should show what are the essential considerations and what are the unessential ones in drawing up the ordinary map.

28–2

In the first place a crossing-over map must correspond to a diagram of pachytene association, if it is during this association that crossing-over takes place (Darlington, 1930 b).

Secondly, the zero point must be the spindle attachment of a chromosome, because at this point identical chromatids are associated (shown in triploid *Drosophila*).

Thirdly, in a ring of n chromosomes n spindle attachments will coincide at zero, from which point the arms will radiate. The simplest example of this is given in the ring of four (Text-fig. 25). This map is more or less applicable to *Pisum* and *Campanula*.
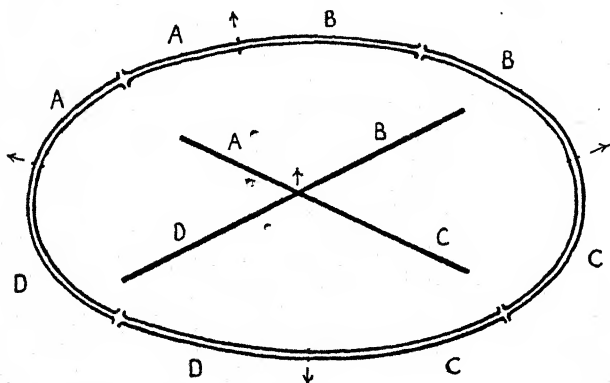
Text-fig. 25. Diagram representing a ring of four chromosomes at diakinesis. Letters show homologous segments which have interchanged at the points of attachment (shown by arrows). This constitution gives the simplest possible type of crossing-over map shown by the cross in the centre (drawn to scale). The point of intersection is the point of zero crossing-over at which all four points of attachment coincide.

Fourthly, the case where the change of homology does not coincide with the spindle attachment cannot be conveniently represented, because the cross-over gametes of the overlap region will be segmentally interchanged, and half of them will be non-viable, thus reducing the cross-over distances in this region to half. And where, as in *Oenothera*, a "complex" or "lethal" system is concerned a higher but uncertain proportion would be non-viable. Such a-lethal segregates as *lutescens* probably arise in this way. These complications cannot properly be considered in relation to the crossing-over map (*vide* Section X).

Fifthly, the fact that segmental interchange only occurs exceptionally in *Oenothera* shows that the point of attachment lies between the two pairing segments, and probably in the differential segment in heterozygotes.

Sixthly, on the basis of the chiasmatype hypothesis as I have defined it (Darlington, 1930 b), and the observation (Section V) that the chiasma frequency is little less than one per pair of segments at metaphase in *Oenothera* and greater than this at prophase, the length of each arm is greater than fifty Morgan units of crossing-over.

These considerations demand a cross-over map in a ring-forming *Oenothera* such as that in Text-fig. 26. I have represented zero crossing-
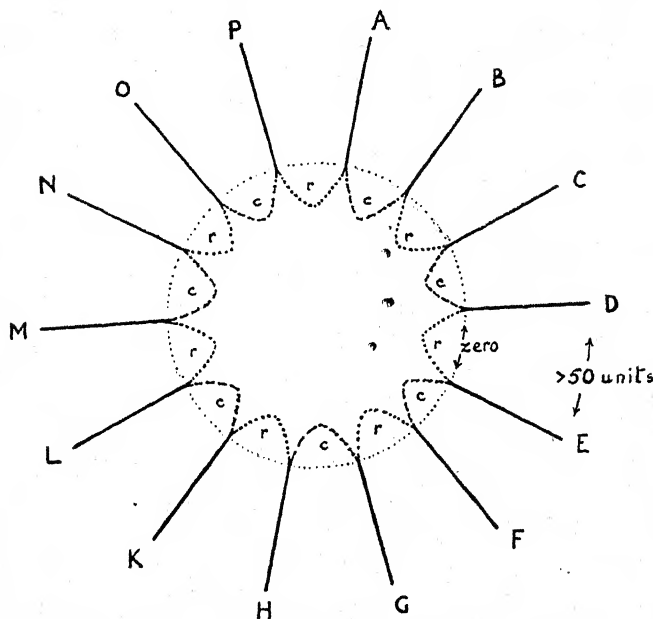
Text-fig. 26. Diagram to represent potential crossing-over map in *Oe. muricata* of the constitution

$$curvans:\ A_cB\quad C_cD\quad E_cF\quad G_cH\quad K_cL\quad M_cN\quad O_cP$$
$$rigens:\ \quad B_rC\quad D_rE\quad F_rG\quad H_rK\quad L_rM\quad N_rO\quad P_rA$$

c represents the differential middle segments of the seven *curvans* chromosomes, r those of the *rigens* chromosomes. Crossing-over occurs between terminal segments with the same capital letters, not between the c and r segments adjacent to these. The lengths of the interior segments are shown equal and likewise those of the exterior segments, but this is arbitrary, for their lengths must be variable following the occurrence of segmental interchange as a result of exceptional crossing-over within the circle, *i.e.* between complex differentials. *Note*: this map corresponds to a constant complex, not to changes of complex with which most crossing-over determinations in *Oenothera* are concerned.

over not as a point but as a circle, because in *Oenothera* all the differential regions have this value and it is important to represent them, (1) in order to show the relationship with pachytene pairing, and (2) because each complex has potential crossing-over values *vis-à-vis* of a different complex or in a homozygous condition.

It is highly improbable that such a map could ever be compiled for *Oenothera*, because the genetic differences in the ring-forming types are confined to the regions in which no crossing-over occurs. The possibility of testing it in principle is offered rather by artificial hybrids with ring formation, such as those in *Pisum*. But several useful deductions can be made from it, and these can be tested.

(i) The maximum crossing-over distance within the ring is the sum of two arms, being no greater whether these arms are in adjoining or remote chromosomes. This is because the crossing-over distance is related to the *radial* pachytene association, not to the *circular* metaphase association.

(ii) The map is in agreement with the conclusion of Renner (1927) that "ein Ringsystem...konnte ganz unabhängiges Mendeln ebenso gut gewahrleisten, wie es die Bildung freier Gemini tut." It shows that free crossing-over will occur freely between factors located distally in the ring chromosomes and the complexes, for their map distance is greater than fifty units apart. This follows from two assumptions: (1) the mean chiasma frequency in each arm is greater than one, for there is usually one at metaphase, and the behaviour of the triploids (Section VIII (1)) shows that it may often follow the terminalisation of two in the diploid; (2) each chiasma follows crossing-over between two chromatids from opposite pairs of the four associated at pachytene (Darlington, 1930 *b*). The observation of free assortment as between the **R** factor and the *velans* and *gaudens* complexes does not therefore prove the **R** factor to be in the free pair of chromosomes in *Oe. Lamarckiana* (cf. Cleland and Blakeslee, 1930).

But one is perhaps less likely to find genetically visible crossing-over taking place within the ring, for the reason that genetic differences are more likely to arise in the middle segments of chromosomes between which I am assuming that pairing (and therefore probably crossing-over) occurs in bivalents, but not in the multiple rings. On the other hand, the evidence that Renner quotes (1928) of crossing-over within rings does not prove crossing-over on the basis of the map. Renner points out that two factors may be inherited independently in plants with a ring of twelve and one bivalent. But, on my hypothesis of crossing-over, a ring-pair with a mean of two chiasmata must be 100 Morgan units long. Two factors might therefore easily be inherited at random, although in the same chromosome pair.

(iii) The map shows diagrammatically another deduction to be derived from the hypothesis, *viz.* that the possibility of an exchange of

whole chromosomes between opposite complexes is absolutely excluded under normal conditions (cf. Section VIII (3) (iv)). The observations which Renner (1928) tentatively suggested might show that it was "gleichgültig ob die Kombinationen in den elterlichen Keimzellen *AB–ab* oder *Ab–aB* gewesen waren" would be incompatible with the hypothesis if they could not be ascribed (I think reasonably) to the alternative which Renner suggests of selection in the course of development.

X. THE BASIS OF MUTATION.

(1) CLASSIFICATION OF CHANGES.

A change (apart from reduction in the number of chiasmata) observed in the configuration of the chromosomes at meiosis in diploid organisms with complete terminalisation of chiasmata, must be due to an interchange amongst the distal segments which are effective in pairing, unless it is due to simple segregation of a homozygous recessive, as in the case of the mutant *ochracea* of *Oe. grandiflora* (De Vries, 1918 *b*; Gerhard, 1929). Whether this segmental interchange takes place in the way I have described (Section VI) or not, there are two types of interchange that must be at once distinguished:

(i) Between chromosomes whose attachments lie on opposite sides of the point of crossing-over or interchange. This will give one new chromatid deficient in a segment (and lacking an attachment), and another with reduplication. Neither chromatid will be physiologically or mechanically *competent* in a ring-forming plant (*i.e.* able to fulfil its functions at meiosis), and we need not consider this type of change.

(ii) Between chromosomes whose attachments lie on the same side of the point of crossing-over. With this type several kinds of effective interchange may be imagined, both in homozygotes and in structural hybrids (with multiple rings). In classifying them I distinguish between "complex" chromosomes within a multiple ring and "non-complex" chromosomes associated only in pairs, and also between "corresponding" segments (lying on the same side of the attachment constriction in the two chromosomes in the ring) and "non-corresponding" segments (lying on opposite sides of the attachment constriction). The term "interchange" is restricted to exchanges of non-homologous segments.

The types of interchange can be discussed under the following heads:

A. Segmental interchange between two non-complex chromosomes.

B. Segmental interchange between a complex and a non-complex chromosome.

C. Segmental interchange between chromosomes in opposite complexes:

    (i) between "non-corresponding" segments;

    (ii) between "corresponding" segments.

D. Segmental interchange between chromosomes within the same complex:

    (i) between "non-corresponding" segments;

    (ii) between "corresponding" segments.

All these types might be expected to occur in *Oenothera* on the two cytological assumptions I have made and substantiated by observation in my material, *viz.* (1) that translocation has taken place freely between the differential middle regions of the *Oenothera* chromosomes, and (2) that segmental interchange takes place as a result of crossing-over between relatively translocated segments. But the expectations are *a priori* so far as genetical considerations are concerned: the effects of the different changes on inheritance and chromosome behaviour, as will be seen, are various but characteristic for each class. Classes must therefore be considered separately. Detailed implications that seem irrelevant to the present problem will be omitted for simplicity.

(2) Description of the mechanism.

A. *Segmental interchange between non-homologous non-complex chromosomes.*

If we represent two pairs of chromosomes in an ancestral homozygous race (of *Oenothera*) AxB, AxB, and CD, CD, x being any small interstitial segment, then by translocation of x to CD we can get a homozygous race of the constitution AB, AB and CxD, CxD. Exceptional crossing-over in a hybrid of these two races, between the small x segments (like that shown in my *biennis* material), will, if the end of x which lies next to A also lies next to C, give rise to two new chromosomes of the constitution AxD and CxB (Text-fig. 27: Parent). These two, segregating disjunctionally from their mates, will form a viable gamete, provided that the reduplication of the small x segment is not lethal. They will meet gametes of four different kinds produced by the hybrid without exceptional crossing-over, if these are viable. Since the conditions of gametic life on the male and female side are on general grounds, to be regarded as having different physiological requirements, and are shown by Renner's work (1919, 1921) to have different genetic requirements, it may be that the new chromosome combination $AxD + CxB$ is only
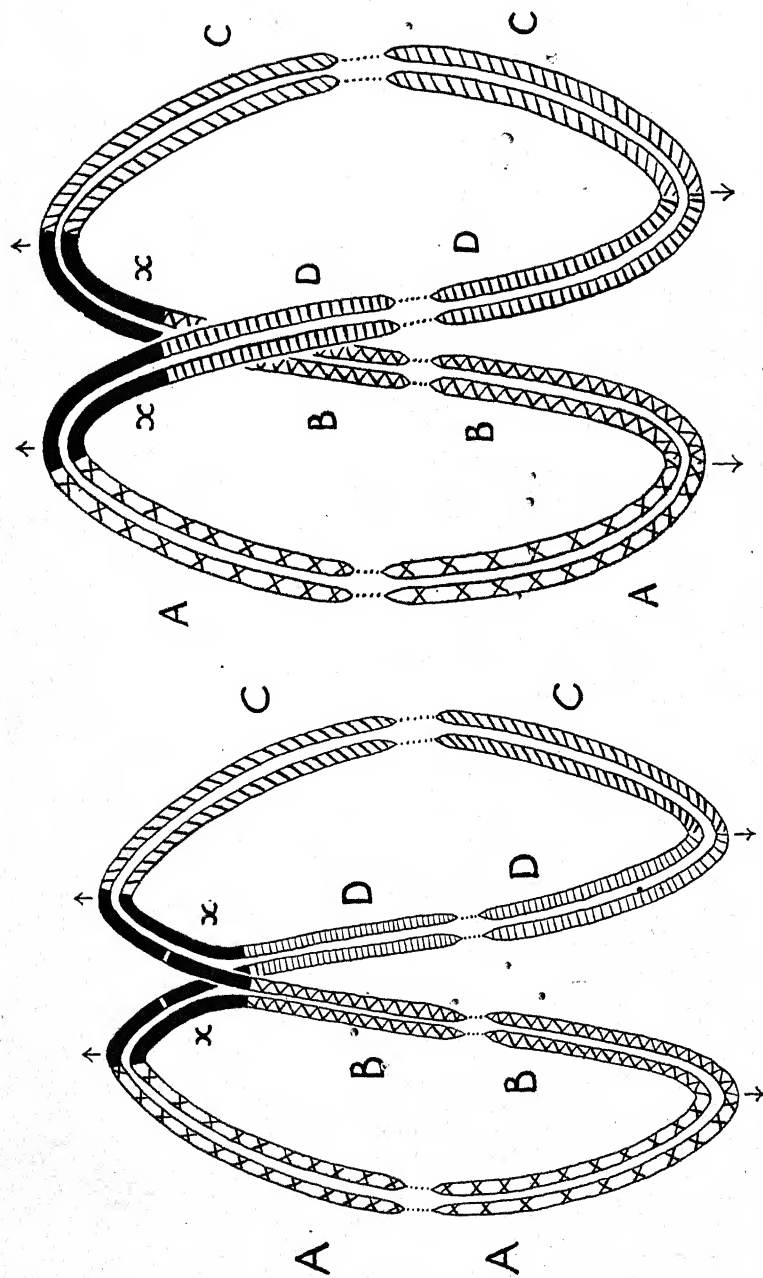
Text-fig. 27. Diagram showing the origin of a "lethal" system, "complex" system, and ring formation by crossing-over between translocated and non-translocated interstitial segments "x." The parent structural hybrid plant of constitution AB, AxB, CD, CxD gives a mutant with a ring of four of the constitution AB, DxC, CD, DxA. The only viable gametes that can be produced by such a system are (i) deficient in x, and (ii) reduplicated in x. They differ in segmental constitution and, meeting will produce the "mutant" structural hybrid with a ring of four. The points of crossing-over in the x segments of two chromatids in the parent are marked by a gap. Terminalisation has taken place and been arrested by the change of homology (Section VI (iii)).

will be the block $\overline{B_{gx}}$ and will include the spindle attachment. This will give an aberrant configuration (cf. Section IX) with a high proportion of irregularities in segregation as observed in *Rosa* (Erlanson, 1931). Secondly, if either new gametic combination is a-lethal, the change will permit the segregation of homozygotes. This is the probable origin of the a-lethal derivatives of complex heterozygotes such as *lutescens* from an *Oe. suaveolens* hybrid (Renner, 1927) and *ochracea* from *Oe. grandiflora* (De Vries, 1918 b; Gerhard, 1929).

(b) The results of this type of exchange between chromosomes unrelated in their pairing segments are more complicated. They are best described by reference to *Oe. Lamarckiana*, in which sufficient data, cytological and genetical, relative to this change have been determined.
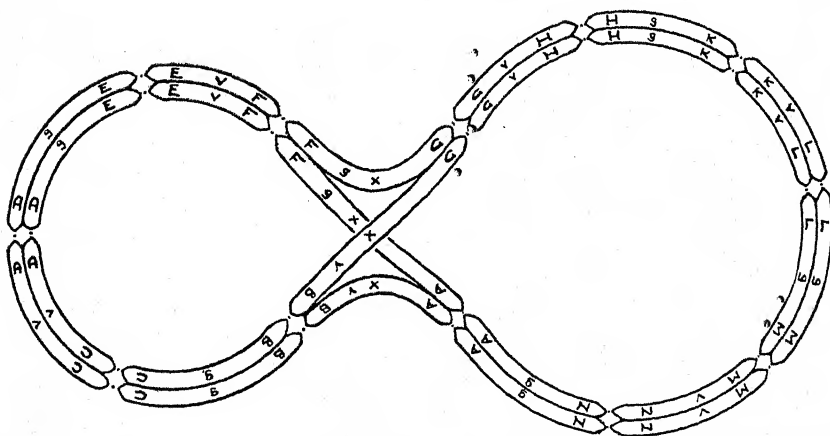
Text-fig. 28. Diagram representing diakinesis configuration in *Oe. Lamarckiana* after chiasma-formation which will give rise to half-mutants (cf. Text-figs. 17–20 and diagram Text-fig. 22). This is described as the figure-of-eight configuration or branched ring (Sections V and VI).

Let the composition of *Oe. Lamarckiana* be:

velans: $(A_{xv}B \quad C_v D \quad E_v F \quad G_v H \quad K_v L \quad M_v N \quad (OP$

gaudens: $B_g C \quad D_g E \quad F_{gx} G \quad H_g K \quad L_g M \quad N_g A) \quad OP)$

If crossing-over takes place within a short interstitial segment "x" common to $A_{xv}B$ and $F_{gx}G$ (giving an interstitial chiasma such as I have described), there will be four chromatids in the metaphase nucleus, including A, B, F and G in four different combinations, viz. $A_{xv}B$, $G_{xv}B$, $G_{xg}F$, $A_{xg}F$. Segmental interchange will have occurred in two of them between A and G (Text-figs. 22 and 28). Segregation (as in quadrivalents giving double reduction) will be non-disjunctional for two of these four

chromatids in any numerically even segregation, and disjunctional for the other two. The position illustrated in the diagram is that for which the segregation is disjunctional in the case of the new combinations, non-disjunctional for the old, which are therefore non-viable. (In the alternative position the new types would be non-viable and no "mutation" could result.) It might be expected in about 50 per cent. of the "figure-of-eight" configurations illustrated.

The effective segregation for the whole complement (*i.e.* neglecting the 50 per cent. of non-viable combinations) will then be:

subgaudens: ($G_{xv}B$　　　C_vD　　　E_vF　　　A_gN　　　M_gL　　　K_gH　　　(*OP*

subvelans:　　　B_gC　　　D_gE　　　$F_{gx}A$　　　N_vM　　　L_vK　　　H_vG) *OP*)

The two new complexes (each occurring in one-eighth of the germ-cells from mother-cells with interchange) will give four new combinations with the two old ones. Extending Renner's (1917) nomenclature I will call the old complexes *velans* and *gaudens*, and the new complexes *subgaudens* and *subvelans* since they are each half-and-half *velans* and *gaudens*.

I
(8) + 3 (2)
{ *velans:*　　　($A_{xv}B$　　　G_vH　　　K_vL　　　M_vN　　　(C_vD　(E_vF　(*OP*

subgaudens:　　　$B_{vx}G$　　　H_gK　　　L_gM　　　N_gA)　C_vD)　E_vF)　*OP*)

II
(6) + 4 (2)
{ *velans:*　　　($A_{xv}B$　　　C_vD　　　E_vF　　　(G_vH　(K_vL　(M_vN　(*OP*

subvelans:　　　B_gC　　　D_gE　　　$F_{gx}A$)　G_vH)　K_vL)　M_vN)　*OP*)

III
(6) + 4 (2)
{ *gaudens:*　　　(B_gC　　　D_gE　　　$F_{gx}G$　　　(H_gK　(L_gM　(N_gA　(*OP*

subgaudens:　　　C_vD　　　E_vF　　　$G_{xv}B$)　H_gK)　L_gM)　N_gA)　*OP*)

IV
(8) + 3 (2)
{ *gaudens:*　　　(B_gC　(D_gE　($F_{gx}G$　　　H_gK　　　L_gM　　　N_gA　(*OP*

subvelans:　　　B_gC)　D_gE)　G_vH　　　K_vL　　　M_vN　　　$A_{xg}F$)　*OP*)

Note: segments acting as a single block in pairing are connected by a vinculum.

One-quarter of the progeny from the "mutated" germ-cells, or one-sixteenth of that from the "mutated" mother-cells, will appear as a particular type of mutant. If the figure-of-eight occurs as I have found in my material in about 2 per cent. of cells, the mutant will appear in 0·12 per cent. of the progeny. Let us consider the genetical observations of mutants in *Oenothera Lamarckiana* with changed configurations.

Oenothera Lamarckiana ((12) + (2)) produces on self-fertilisation about 40 per cent. of good seeds (Renner, 1916, 1917, p. 138). The remaining 60 per cent. are empty (*gaudens-gaudens*) or with rudimentary embryos (*velans-velans*, cf. Renner, 1929, p. 84 and 1916). The good seeds give about 98 per cent. of plants like the parent (*velans-gaudens*) and

about 2 per cent. of mutant forms, mostly with aberrant chromosome numbers. Amongst those with the normal number is the half-mutant *Oe. rubrinervis* ((6) + 4 (2), Cleland, 1925) which makes up about 0·1 per cent. of seedlings or 0·04 per cent. of potential zygotes (cf. expectation above) (De Vries, 1919 *a*). The half-mutant has about one-quarter non-viable offspring, one-half or more like itself and one-quarter or less of the true-breeding (95 per cent. fertile) mutant *deserens* (De Vries, 1919 *a*; 7 (2), Cleland, 1925).

Oenothera Lamarckiana is described as composed of two complexes *velans* and *gaudens*, *Oe. rubrinervis* of two complexes *paenevelans* and *subvelans*, and the mutant *deserens* of two *subvelans* complexes (Renner, 1925). Genetical analysis of the half-mutant *rubrinervis* has led to the following conclusions—"dass *subvelans* einem Faktorenaustausch zwischen *velans* und *gaudens* die Entstehung verdankt" (Renner, 1917), and "der Komplex *paenevelans* ist von *velans* in den meisten Verbindungen nicht zu unterscheiden.... Das einzige Mittel zur sicheren Unterscheidung von *velans* und *paenevelans* ist die Verbindung mit *subvelans*" (Hoeppener and Renner, 1929).

It will be seen that the second of the four possible mutant types agrees with the description of *Oe. rubrinervis*.[1] We may therefore assume the following relative formulae:

Oe. Lamarckiana (12) + (2):

velans: $(A_{xv}B \quad C_vD \quad E_vF \quad G_vH \quad K_vL \quad M_vN \quad (OP$

gaudens: $B_gC \quad D_gE \quad F_{gx}G \quad H_gK \quad L_gM \quad N_gA) \quad OP)$

Oe. rubrinervis (6) + 4 (2) (half-mutant):

paenevelans: $(A_{xv}B \quad C_vD \quad E_vF \quad (G_vH \quad (K_vL \quad (M_vN \quad (OP$

subvelans: $B_gC \quad D_gE \quad F_{gx}A) \quad G_vH) \quad K_vL) \quad M_vN) \quad OP)$

Oe. deserens (7 (2)) (full-mutant):

subvelans: $(B_gC \quad (D_gE \quad (F_{gx}A \quad (G_vH \quad (K_vL \quad (M_vN \quad (OP$

subvelans: $B_gC) \quad D_gE) \quad F_{gx}A) \quad G_vH) \quad K_vL) \quad M_vN) \quad OP)$
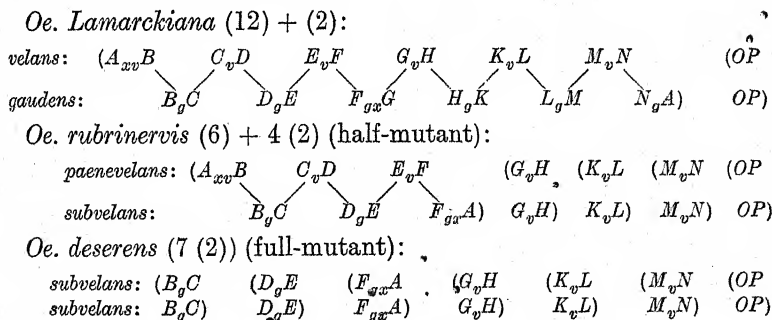
A second half-mutant of *Oe. Lamarckiana*, *erythrina*, we may consider, arose by crossing-over in the *x* segment if we assume the *fragilis* factor to be located there (De Vries, 1919 *b*). A third half-mutant, *rubrisepala*, of Heribert-Nilsson (Håkansson, 1930 *b*) we may consider arose

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[1] There is the alternative possibility that crossing-over took place between segments arranged $A_{vx}B$ and $F_{xg}G$ to give new chromatids $A_{vx}F$ and $B_{xg}G$. There is no evidence to discriminate between these two hypotheses, but this type of crossing-over would give in *rubrinervis* continuous homology in three segments (as in types I and IV in the first hypothesis), viz. A_{vx}, which would yield chiasmata interstitially between chromosomes associated terminally in the other arm, and these have not so far been observed.

by crossing-over between chromosomes one pair closer than in the case of *rubrinervis*. A fourth, *Oe. problandina* (De Vries, 1917, 1918 *a*) gives a full mutant, *Oe. blandina*, in the same way as the others.

This hypothesis agrees with the observations of the origin and behaviour of half-mutants in the following respects:

(1) Frequency of half-mutants should be constant, being determined by crossing-over between given lengths of chromosome.

(2) Type of mutant should be constant, because the point of crossing-over is fixed within narrow limits and between similar segments.

(3) The constitution of the two complexes of the mutant should be (i) unchanged, and (ii) a mixture of the two parental complexes. *Note:* the occurrence of the very slight change between *velans* and *paenevelans* can be understood as the result of crossing-over which can now occur regularly between the two *x* segments. The differential region of *velans* is reduced by *x* which becomes part of a pairing segment in *rubrinervis*.

(4) The chromosomes in the mutant should form a ring of six and four pairs.

(5) The new half-and-half complex succeeds while its complement, *subgaudens*, fails. It may therefore not expectedly occur homozygous in the second generation owing to its lethal factors having passed to the complementary complex. Or we may say: since *subvelans* is more viable than *subgaudens*, then it may well be more capable of homozygous existence than the almost equally inviable *velans* and *gaudens*.

(6) Since exceptional crossing-over should be easier in trisomic and triploid forms, it is not surprising that trisomics have given characteristic half-mutants not found in the progeny of diploid *Lamarckiana*, and in a higher frequency. In this way *problandina* has arisen from a cross *lata* × *semi-lata* (De Vries, 1917). Similarly six mutants occurred in 100 progeny of the trisomic *Oe. nitens*; amongst these was the half-mutant *distans* (De Vries, 1923). The origin of these mutants cannot be described exactly as in other cases of changed configurations because the parental configurations are not known. But there is no reason to doubt that the mode of origin is in principle the same.

(ii) *Between corresponding segments.*

Half-mutants will arise from segmental interchange between segments in opposite complexes that do not correspond in position, *i.e.* between *A* and *E*, not between *A* and *D*. It is, on the other hand, impossible to have mutation in the diploid from crossing-over and interchange between non-homologous segments of opposite complexes which

correspond in position, because in this way segments are brought into the same chromosome, from which regular disjunction of homologous segments in the ring is impossible. Such segmental interchange could give mutants in the second generation through a trisomic in the first if double non-disjunction accompanied the interchange. I have suggested (1929 a) that Cleland's *oblonga* was a trisomic of this type.

D. *Segmental interchange within the same complex.*

(i) *Between non-corresponding segments.*

This kind of interchange will give no change in the size of the ring and no change in the genetic properties of the complex. It will merely reverse the position of one or more chromosomes in the ring and the order of their segments. Its occurrence will therefore be incapable of demonstration except from comparison of the behaviour of the changed and unchanged types in hybrids with a second species.

(ii) *Between corresponding segments.*

Take a ring, with complexes α and β, of the constitution:

$$(A_{a\alpha}B \qquad C_a D \qquad E_{a\alpha}F \qquad G_a H$$
$$B_\beta C \qquad D_\beta E \qquad F_\beta G \qquad H_\beta A)$$
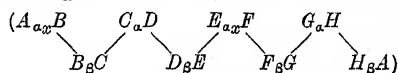
Interchange of the segments B and F will give only one new viable (disjunctional) arrangement with one new type of gamete, which will give, with its complementary complex, the zygote

$$(A_{a\alpha}F \qquad G_a H \qquad \qquad (C_a D \qquad E_{a\alpha}B$$
$$F_\beta G \qquad H_\beta A) \qquad \qquad D_\beta E \qquad B_\beta C)$$
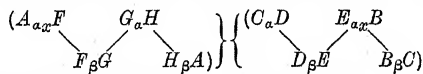
(With its unchanged sister-complex it will always give a ring of four and the rest of the chromosomes in pairs. The zygote would probably be lethal being nearly homozygous.) The mutant gamete will have changed mechanical (*i.e.* pairing) properties but unchanged physiological properties, so that mutant offspring will be indistinguishable except in their segregation. They will now have two pairs of complexes capable of random segregation. If unlike *Oe. biennis* $((8) + (6))$ they are capable of random combination to give viable gametes, and if any of the four new complexes is capable of homozygous existence side-by-side with a heterozygous or homozygous sister-complex, then mutant segregates will appear in the second generation. Let the two pairs of alternative complexes be Aa and Bb, then the type combination is $AaBb$ and the (mechanically possible) expected zygotic types will be in the random proportions:

$1\,AABB : 1\,AAbb : 1\,aaBB : 1\,aabb : 2\,AABb : 2\,AaBB : 2\,Aabb : 2\,aaBb : 4\,AaBb.$

Since it is probable that the new combinations will be less successful than the old, both zygotically and gametically, no mutant will be likely to appear in the second generation in a higher proportion than half that of the parent type (*AaBb*). Eight mutant types are possible; six only, if the mutant gametes (*Ab* and *aB*) are viable only on one side. But the occurrence of the interchange will lead to a lengthening of the segments capable of pachytene pairing and crossing-over in the neighbourhood of the point of interchange (as in type C (i) (*b*)). Portions of the two complex regions next to the two newly opposed pairing segments may be homologous but dissimilar, and therefore give segregation. Such segregation in the first and later generations will lead to an increase or decrease in the proportion of mutants given by *AaBb* in successive generations as a result of the changes in the constitution of the complexes or, as one may say, by crossing-over of lethal factors.

I conclude that the following conditions will be characteristic of mutation following this type of change:

(i) It will occur in races having one complex with a reduplicated segment in different chromosomes.

(ii) It will be less regular in original occurrence than the half-mutant type, for it will depend on randomness of selection in the population of parents instead of in the larger population of offspring.

(iii) The mutating individual will be indistinguishable from non-mutating individuals, except in its offspring.

(iv) The mutating individual will yield a proportion of mutant segregates of not more than eight pairing types, or six if the mutated types of gamete only function on one side. The proportion will vary in different mutating types according to the relative gametic and zygotic viability of new and old types.

(v) The mutating individual will have two chromosome rings (simple or multiple) corresponding to one ring of its non-mutating parent.

(vi) Its mutants will have homozygous pairs corresponding to *one* or *both* of the heterozygous rings. The first classes will be the more numerous. They will yield the second on selfing. The second will breed true to the absence of rings.

(vii) The mutants will therefore be partly or wholly homozygous and will never yield the type on selfing.

(viii) The mutating individual will institute an ever-sporting race having the same pairing configuration as itself, and it may give higher proportions of mutants in later generations.

(ix) The mutants will give their own type and the parental type in

crosses with their parent, but, if the new sub-complexes are heterogamous in the ever-sporting race, the mutants will give different results in reciprocal crosses.

(x) The partially heterozygous mutants will be less fertile than their parents if the mechanism of heterogamy is no longer reciprocal.

Note: The type of segregation mechanism imagined in the eversporting type has often been produced in *Oenothera* by hybridisation (Rudloff, 1930 *et al.*).

These conditions appear to be fulfilled genetically by the appearance of the mass mutants, *semialta, debilis, rigida* and *bilonga* of *Oe. Reynoldsii* (Bartlett, 1915 *a*; La Rue and Bartlett, 1917). Whether they are fulfilled cytologically is not yet known. The mass mutant *setacea* of *Oe. pratincola* (Bartlett, 1915 *b*) might also be derived in the same way, but more probably (cf. Blanchard, 1929) like the "mass mutant" *Oe. grandiflora ochracea* (De Vries, 1918 *b*) it falls in category C (1) (*a*), while the "mass mutants" *deserens, blandina* of *Oe. Lamarckiana* (De Vries, 1917, 1918 *b*, 1919 *a et al.*) fall in category C (1) (*b*).

If the *Reynoldsii* mutations fall in this class, then *rigida* and *bilonga* must be regarded as the full mutants corresponding to half-mutants, *semi-alta* and *debilis*. *Bilonga* should bear the same relationship to *debilis* that the full mutant *deserens* bears to the half-mutant *rubrinervis*. In both cases, we may note, the secondary (homozygous) segregate is more vigorous than the primary form.

One observation in these experiments is not directly anticipated. A *typica* form in the first mutant family (F_3) of *Oe. Reynoldsii* (Bartlett, 1915 *a*) gave a uniform progeny. This means (in terms of the present hypothesis) that its parent in the first segregating generation has suffered reverse interchange. Such an occurrence should on the simplest expectation have the same (unknown but presumably rare) frequency as the original interchange. The observation is therefore surprising as a chance but anticipated in its character.

Notes. (i) It is improbable that fragmentation plays any part in mutation in *Oenothera*. Observations of fragmentation at somatic divisions are probably due to misinterpretation of constrictions (*vide* Section IV). Observations of fragmentation at meiosis are probably due to misinterpretation of interstitial chiasmata (*vide* Section V).

(ii) These mutations as a result of exceptional crossing-over in a "gametically" self-sterile organism are analogous to the segregation in new ratios as a result of exceptional crossing-over in the region of self-sterility factors in a "relationally" self-sterile organism (cf. Brieger, 1930).

(3) MUTATION: STRUCTURAL OR CHEMICAL CHANGE?

It would seem that all permanent hereditary changes yet identified in *Oenothera* fall into the categories described above (apart from tetraploidy, which has played no part in the evolution of the *Onagra* section). All these changes are of the nature of changes in arrangement and proportion of the hereditary materials. They are changes in gross structure rather than changes in the molecule. They differ in this respect from most of the changes identified in *Drosophila*, and they also differ in that they are of proven value to the organism, which very few of the changes in *Drosophila* are. But changes in the molecule alone can give rise to the qualitative differentiation which we observe within the hereditary material of all organisms. The distinction between *molecular* changes and *structural* changes is therefore an important one. The first (of the *Drosophila* type) are of necessity the primary basis of mutation, the second (of the *Oenothera* type) are a secondary basis of greater immediate importance in certain organisms. The two are complementary agents of evolutionary change.

Apparently, the molecular changes are the more frequent but are rarely advantageous, usually disadvantageous, in their effect. The structural changes are less frequent, but their secondary results in crossing-over in hybrids are potentially frequent and often advantageous. Their relative importance as immediate agents of evolutionary change must therefore be different according to the frequency of hybridisation.[1]

It has become usual to speak of "genes" as units of variation and at the same time to speak of chromosomes as made up of genes. But, in view of this distinction, it is appropriate to ask whether, if any one particle can change in two ways, internally (chemically) and externally (structurally), the theory which relates genetic differences with material particles and describes both as genes (Morgan, 1925) can be validly applied to all organisms. It will be seen that in the present study where we are concerned primarily with structural changes, any mendelian formula for translating genetic conceptions into cytological terms and *vice versa* would be cumbrous and indirect, while the gene formula, with its special implications, would be actually misleading. In the present study I have therefore, as far as possible, avoided borrowing (by translation) conceptions justified by genetic experiment in other fields but inadequately verified in *Oenothera*.

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[1] I have argued earlier (1929 *d*) that hybridisation must operate against the survival of structural changes (such as fragmentation) by reducing fertility.

XI. THE FREQUENCIES OF THE PAIRING TYPES AND THEIR EVOLUTIONARY MEANING.

(a) IMMEDIATE CONCLUSIONS.

The hypothesis that the ring-forming species of the *Onagra* group are complex structural hybrids is related to five assumptions affecting the possible configurations of the chromosomes at meiosis in these species, viz.:

(i) There are fourteen distal segments of seven chromosomes with specific pairing properties.

(ii) Every viable haploid gamete (with seven chromosomes) contains these fourteen segments.

(iii) Every zygote therefore contains a pair of each of the segments.

(iv) Gametes may occur with any possible association of specific segments in pairs to make the ends of the seven chromosomes.

(v) There are fifteen possible chromosome configurations in regard to the numbers of rings and the number (always even) of chromosomes in each ring.

Appendix II gives a list of races or potential races of *Oenothera* (*i.e.* diploid forms) arranged under their type of pairing. Doubtless some that are given different names are really the same type, but obvious cases of this kind (such as simple cross-over mutants) I have excluded. The races are arranged under their pairing type in the order of its random frequency as calculated by Prof. Haldane (Appendix I). I have given references so that the method of classification may be critically examined.

The list may give us information in two directions.

(i) The relationship of frequency of total forms investigated to that expected with chance association of segments, such as would be found if segmental interchange had proceeded at random and without selection for an indefinite period. (ii) The relationship in frequency of forms existing under natural conditions to that of forms owing their origin to artificial hybridisation and not subject to the full severity of natural selection.

The classification into species, mutants and hybrids is arbitrary in a few cases. The class "mutants" includes the types discussed in the last section which have a changed configuration. They can all be described as segregates *sensu lato*.

The list is summarised in the following table:

Configuration type	Relative random frequency	Species	"Mutants" or segregates of species	"Hybrids"	Derivatives or segregates of hybrids	Totals
				Observed frequency		
1. (14)	23,040	12	—	15	—	27
2. (12) + (2)	13,040	5	—	16	—	21
3. (10) + (4)	8,064	—	—	5	—	5
4. (8) + (6)	6,720	2	—	2	—	4
5. (8) + (4) + (2)	5,040	—	—	1	—	1
6. (10) + 2 (2)	4,032	—	—	11	—	11
7. 2 (6) + (2)	2,240	—	—	—	—	0
8. (6) + 2 (4)	1,680	—	—	1	—	1
9. (6) + (4) + 2 (2)	1,680	—	—	3	1	4
10. (8) + 3 (2)	840	—	4	1	1	6
11. 3 (4) + (2)	420	—	—	—	—	0
12. 2 (4) + 3 (2)	210	—	—	3	—	3
13. (6) + 4 (2)	140	—	3	5	2	10
14. (4) + 5 (2)	21	1	1	8	—	10
15. 7 (2)	1	4	6	3	4	17
Totals		24	14	74	8	120

We must consider the classes in turn in relation to two unknown factors: the amount of interchange that has occurred and the effect of natural selection. Of the second we have evidence from other sources.

(i) *The Hybrids* give the best indication of segmental relationships in the group, but they are a selection of viable combinations. They therefore include a disproportionately high number of the large-ring forms which are the least homozygous. I conclude therefore that a random selection of combinations would show a much higher frequency of low ring types, *i.e.* a frequency disproportionate to the expectation with the chance association of segments in *Oenothera*. This probably means that *Oenothera* has produced only a small fraction of the possible interchange types.

(ii) *The Species* show that amongst those which have a ring-mechanism a disproportionately high number occur with a ring of fourteen. Clearly the transitional stages are not favoured by natural selection. They are mechanically and physiologically less perfect devices. Those with seven bivalents have presumably never adopted a ring mechanism, while *Oe. franciscana* is presumably in the first stage of development or a mutant of the a-lethal D (ii) type.

(iii) *The Mutants* all fall in the more homozygous classes because this is the only direction in which change of configuration is possible. They therefore afford no indication of the range of variation in the pairing types.

(b) GENERAL CONSIDERATIONS.

The complex-heterozygote *Oenothera* species are hybrids in that each zygote is made up of two dissimilar gametes. The dissimilarity of the gametes is shown genetically by the production of twin hybrids (cf. Renner 1929, footnote, p. 85), and cytologically by the different pairing properties of the chromosomes in the ring (on my hypothesis).

Two opposed explanations of the origin of the complex heterozygote have been advanced:

(i) They arise by inter-specific hybridisation (Renner, 1917, pp. 280–85).

(ii) They arise by inherent mutability of their ancestors (De Vries, 1918 *a*, p. 309). This means that the process has involved what Lotsy has described as "internal hybridisation."

The distinction between these two hypotheses does not concern the quality or mode of origin of differences but merely their distribution. Now the complex heterozygote has differences of two kinds: those which distinguish the arrangement of the chromosome ends, and those which distinguish the genetic properties of the differential segments. The first are the easier to study with regard to their origin, and we can distinguish between the two hypotheses by their application to this problem diagrammatically. The two opposed assumptions would be:

(i) Six occurrences of segmental interchange in derivatives of a common homozygous ancestor giving six different homozygous types which on crossing would yield a type with a ring of fourteen.

(ii) Cumulative interchange with self-fertilisation which in six steps would yield a ring of fourteen.

A third extreme hypothesis is that two homozygous forms (with seven pairs) might arise by cumulative interchange, which, when crossed, would immediately yield a ring of fourteen. This hypothesis predicates chances which are unlikely because they are not increased, as in the second type, by the effect of natural selection.

In my original study I followed the second hypothesis, and I think this is the more likely for two reasons. (i) It is easier to imagine the secondary adaptations of the complexes to be developed in association with the changes that distinguish the complexes, than to imagine that the complexes are thrown together by hybridisation after the structural changes have taken place, for natural selection will favour the first result but not the second. (ii) It is probable that segmental interchange is correlated with the origin of the balanced lethal system (Section X). It

is not easy therefore to suppose that homozygous forms are segregated from the interchange-mutants, and that these afterwards, by hybridisation, restore the ring. For hybridisation in itself cannot introduce a system of balanced lethals. Nor will it (by chance) give the high proportion of forms found with a ring of fourteen.

Observations such as the occurrence of the *albicans* complex common to *Oe. biennis* and *Oe. suaveolens* point strongly (as Renner remarks) to hybridisation being responsible for certain heterozygote combinations.
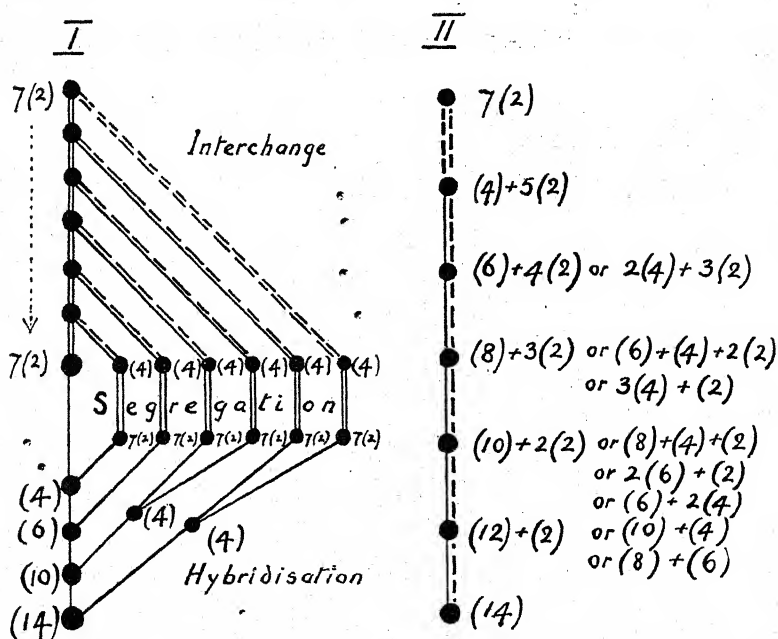
Text-fig. 29. Diagram representing opposite hypotheses with regard to the method of origin of a structural hybrid with a ring of 14 chromosomes by segmental change from an ancestor with 7 pairs. The circles represent zygotes, the lines gametes. A broken line represents a gamete in which a new segmental interchange has occurred. With both methods types seven such types need to be formed.

But such hybridisation must be regarded as secondary, and not responsible for the constructive changes which have given rise to the ring mechanism. In several cases there is reason to suppose that this secondary hybridisation has taken place since the introduction into Europe. The specialisation of the functions of segments would facilitate this process (Section VIII). Moreover, where two hybrids are formed between gametes equally distinct physiologically, one having a potential ring

of fourteen and the other a potential seven pairs, the former will be vastly more fertile than the latter. Ignoring non-disjunction and crossing-over in both cases, the former will produce two kinds of gametes, each identical with a viable and functional parental type; the latter will produce 2^7 types of gametes of which two will be of the viable and functional parental types, the rest (according to the rule, which we may make, that chance combinations are less successful than those which have survived natural selection) will be less successful. In most interspecific hybrids they are non-viable. The ring form will therefore be a function of 2^{n-1} times as fertile as the pairing form where n is the haploid number. I referred to this principle in general terms in my earlier study (1929 a). It follows that, while structural differences are probably the chief causes of sterility in hybrids between species, when these are combined with regular interchange to give ring formation, the disability is removed: segregation is limited and fertility preserved. Most interspecific hybrids have complex differences like the *Oenothera* species, but they have not the ring mechanism to preserve both hybridity and fertility.

I conclude therefore that the evolutionary course of events has lain somewhere between the two artificial extremes which I have chosen for examples.

The constructive changes, if my view is accepted, depend directly and alone on the occurrence of translocation and crossing-over. A comparison of the chromosome complements of related species shows that the capacity for such changes is inherent in the hereditary material of all organisms. The capacity for combining them to produce the *Oenothera* mechanism however depends on certain mechanical, crudely morphological and physiological properties, such as (i) the possession of a low diploid number, with median or submedian attachment constrictions; a higher number or subterminal constrictions would give greater irregularity of segregation. (ii) An unusual regularity of terminalisation of chiasmata. (iii) A high seed production. (iv) Extreme sensitiveness of pollen grains and megaspores to changes in chromosome constitution, or, we may say alternatively, sharp qualitative differentiation of the chromosomes, for we perhaps ought to consider extreme "mutability" as an indication rather of the degree of visibility of changes than of their frequency.

XII. THE *OENOTHERA* HYPOTHESIS.

It is now possible (and necessary) to restate in more precise terms the hypothesis that I put forward three years ago with regard to the behaviour and relative structure of the chromosomes in the ring-forming *Oenothera* species. It will be noticed that in every relevant detail this hypothesis follows from the general theory of meiosis that I hold to be universally applicable (1929 *b* and *c*, 1931 *b*). *Oenothera* is not an exception to the principles of this theory but, instead, a useful example of its application.

The hypothesis is as follows:

1. A plant with ring-forming chromosomes is derived from the union of gametes which differ in the structure of their chromosomes in such a way that each chromosome in the ring is made up of two distal segments (*i.e.* lying between the spindle attachment and the end of the chromosome) which correspond, in having the same linear arrangement of materials, with the distal segments of two different chromosomes in the opposite gametic set.

2. There is a "differential segment" in each chromosome, between the two distal segments of known homology, which does not continue the homologies of the distal segments because it does not correspond in linear arrangement with the same segment in the opposite complex. It includes the spindle attachment.

3. The seven differential segments of one gamete determine the physiological characteristics of the Renner complex and the lethal factor system. The fourteen distal segments determine by their arrangement the mechanical characteristics of the complex (*i.e.* its properties of pairing and segregation) for the following reasons.

4. Pairing takes place between the corresponding distal segments at a pachytene stage of meiosis which is normal except that the differential segments, dissimilar in linear structure, remain unpaired.

5. At diplotene, chiasmata are formed at random in the paired segments and then terminalised, so that at diakinesis all association is by terminal chiasmata. Where each of the paired segments has formed one or more chiasmata, the result is a ring. Where one or more pairs of segments have failed to form chiasmata the ring is replaced by a chain, or by two or more chains, just as in simple pairing of the same type a ring pair is replaced by a rod pair.

6. The multiple ring, as in comparable cases in polyploids, usually arranges itself disjunctionally on the spindle at metaphase, because,

presumably, repulsion is greatest in the axis of the spindle and is between attachment points that are nearest. Chromosomes with corresponding segments therefore pass to opposite poles.

7. The origin of ring formation and all effective mutation in *Oenothera* can be expressed in terms of phenomena understood in other organisms. Mutations can be classified as follows:

(*a*) Changes in chromosome number: (1) trisomy, (2) triploidy, (3) tetraploidy (*Oe. Lamarckiana, Oe. biennis, Oe. grandiflora, Oe. pratincola*, etc.).

(*b*) Changes due to crossing-over in complex permanent heterozygotes between homologous *terminal* segments (*i.e.* not involving segmental interchange), *e.g. Lamarckiana nanella* (Renner, 1925, p. 137), *biennis sulfurea* (Håkansson, 1926, p. 287), and many others.

(*c*) Changes due to crossing-over in complex permanent heterozygotes between homologous *interstitial* segments in the differential regions (proximal to non-homologous terminal segments).

(1) Crossing-over between *non-corresponding* segments of members of opposite complexes, giving:

(i) a-lethal segregates, *e.g. lutescens* from *Oe. suaveolens* (De Vries, 1918 *c*, Renner, 1927); or

(ii) primarily half-mutants, *e.g. Lamarckiana rubrinervis* (Darlington, 1929 *a* and above), *erythrina* (Davis, cf. Cleland, 1928); and secondarily a-lethal segregates such as the "full mutants" of *Oe. Lamarckiana*.

(2) Crossing-over between *corresponding* segments of members of the same complex, giving rise to "mass mutants," *e.g. Oe. Reynoldsii* (Bartlett, 1915 *a*).

XIII. NOTE ON DEFINITIONS AND ABBREVIATIONS.

A Renner *complex* is a gametic genotype transmitted in a particular race as a unit in inheritance and associated with seven chromosomes.

A *simple ring* is a ring of two chromosomes. It may be heterozygous or homozygous. A *multiple ring* is a ring of more than two chromosomes at meiosis. It is always heterozygous. In a diploid the number of chromosomes in a ring is always even.

A *complex chromosome* is one taking part in a multiple ring.

A *complex differential* is the part of one complex in which it constantly differs from the complex with which it is for the time being associated. It is localised in the middle segments of the chromosomes (Section VIII) which are the *differential segments*.

A *chiasma* is an observed exchange of partner amongst four chromatids associated in pairs. It is formed interstitially at diplotene, and is determined by genetic crossing-over (*i.e.* exchange of linear association) between two of the chromatids (Darlington, 1929 *c*, 1930 *b*).

A *terminal chiasma* is the separate association of the two ends of the chromatids of one chromosome with the two ends of the chromatids of another. It results at metaphase from the movement towards the ends of the chromosomes of one or more chiasmata formed interstitially (Darlington, 1929 *c*, 1931 *a*, *b* and *c*).

Segmental interchange is an exchange of linear association between non-homologous elements and may result (*vide* Section VI) from genetic crossing-over between homologous elements intercalated between non-homologous elements. I have not used the term (as Belling has) to include ordinary genetic crossing-over (Belling and Blakeslee, 1924, 1926).

Rings are indicated both in structural formulae and configuration formulae by parentheses, thus: (10) + 2 (2) means a ring of ten and two rings of two (*i.e.* two simple pairs). (*AB . AB*) means a ring pair and (*AB–BC–CD–DA*) means a ring of four.

XIV. SUMMARY.

1. New cytological observations on *Oenothera* are shown to demonstrate (*inter alia*) parasynapsis and genetic crossing-over at chiasmata as the means of segmental interchange.

2. An attempt is made to show that in the hybrid species of *Oenothera*:

(i) The observed *chromosome behaviour* is predictable by analogy with other organisms which have been investigated in greater detail.

(ii) The observed *hereditary behaviour* is predictable by analogy with other non-hybrid organisms in which the several complications can be examined separately, on the assumption of a certain type of structural hybridity. This assumption is a translation in terms of chromosome material of the hypotheses which De Vries, Renner, Muller, Oehlkers and others have made in terms of genetic factors or factor systems.

3. The assumption enables one to define the system of inheritance and the method of mutation with a precision necessary for any further analysis of these phenomena.

THE MATHEMATICAL THEORY OF CHROMOSOME RINGS.
By J. B. S. HALDANE.

Consider a plant with two sets of n chromosomes. We have to solve three different probelms: (a) How many pairing types are there? In this enumeration a configuration such as a ring of six and four bivalents is only counted once, no matter how it is made up. (b) How many gametic and zygotic types are there in all? Here we group together all chromosomes whose ends are identical, even if their middle parts differ, since it is the ends which determine the structure of the rings. (c) How many zygotic types belong to each of the pairing types? Problems (a) and (b) can be solved with comparative ease for any value of n. (c) is more complicated.

Number of pairing types.

The $2n$ chromosomes can be associated either in pairs or in rings of 4, 6, 8 and so on. Clearly the number of ways in which $2n$ things can be divided up into groups of even number is the same as the way in which n can be divided into groups of even or odd number. Thus 5 can be divided up as follows: $1 + 1 + 1 + 1 + 1, 2 + 1 + 1 + 1, 2 + 2 + 1, 3 + 1 + 1,$ $3 + 2, 4 + 1, 5,$ i.e. in 7 ways. To the partition $3 + 1 + 1$ corresponds a ring of 6 and 2 bivalents.

The function of n required is the well-known partition function $p(n)$, meaning the number of partitions of n into an unrestricted number of integral parts of unrestricted magnitude. The method of calculating this function, first discussed by Euler, is given in text-books of algebra. Its values up to $n = 12$ are given in Table I. A full account and tables up to $p(200)$ are given by Hardy and Ramanujan (1918).

Total number of gametic and zygotic types.

The $2n$ chromosome ends present in a gamete may be associated in pairs in a number of ways which we shall call $g(n)$. This is clearly the total possible number of gametic types. We represent the ends by $2n$ letters, to be arranged in pairs in n chromosomes. We can arrange these letters in $\lfloor 2n$ orders. But the order of the n chromosomes is irrelevant. So we must divide this number by $\lfloor n$. Also the order in each individual chromosome is irrelevant. So we must again divide by 2 for each chromosome, i.e. by 2^n. So

$$g(n) = \frac{\lfloor 2n}{2^n \lfloor n} = 1.3.5.7.....(2n-1).$$

This function is tabulated in Table I. $g(7) = 135,135$. Any of these types united with itself or any other gives a zygotic type. The total number of zygotic types is thus $\frac{1}{2}g(n)(g(n)+1)$. For $n=7$ this number is 9,130,801,680.

Distribution of the zygotic types among pairing types.

This must be worked out in detail for each value of n. Actual figures are only given for $n=7$, the case exemplified by *Oenothera*. Consider any of the 135,135 gametic types. If this is associated with the same gametic type it gives 7 bivalents, if associated with any other, it gives one or more rings. Calling our gametic type

$$AB \quad CD \quad EF \quad GH \quad KL \quad MN \quad OP$$

a ring of 4 is formed if it meets another gamete in which an exchange has occurred between any two chromosomes, *e.g.* a gamete similar except that it contains EG and FH instead of EF and GH. Clearly there are $\frac{7 \times 6}{2}$ or 21 ways of choosing a pair of chromosomes whose ends are to be exchanged, 35 ways of choosing 3 chromosomes to be altered so as to constitute a ring of 6, and so on. Similarly to make a ring of 6 and a ring of 4 we choose 3 in 35 ways and then 2 out of the remaining 4 in $\frac{4 \times 3}{2}$ or 6 ways, giving a total of 210 ways. If however we are choosing constituents for 2 or 3 rings of equal number, it is clear that the order in which the rings are chosen is irrelevant. We must therefore divide the number of ways of choosing the ring components, as calculated above, by 2 or 6 as the case may be.

We next consider the number of arrangements within a ring of $2m$ chromosomes. The $2m$ pairs of ends can be arranged in any cyclical order, giving $\frac{1}{2}\lfloor 2m-1$ arrangements (since orders which are exact reverses of one another are equivalent). But we are only concerned with rings containing one particular arrangement of half their chromatin, *e.g.* CD, EF, GH. There are $\frac{\lfloor 2m}{2^m \lfloor m}$ such arrangements, for the reason pointed out above.

Each of these occurs in $2 \times \frac{1}{2}\lfloor 2m-1 \div \frac{\lfloor 2m}{2^m \lfloor m}$ or $2^{m-1}\lfloor m-1$ different kinds of ring, counting reciprocals separately. Hence for each ring of $2m$ chromosomes we must multiply the number found in the last paragraph by this expression. This multiplier is twice the number $r(n)$

tabulated in Table I. The resulting number must be divided by two, since each zygote is made from two gametes, and we do not distinguish between zygotes XY and YX.

The results of such calculations for $n = 7$ are given in Table I. An example will show the method of calculation. There are $3.5.7.9.11.13$, or $135,135$ gametic types. The symbol $2 \times (6) + (2)$ represents a zygote with 2 rings of 6 chromosomes and 1 bivalent. The constituents of the first ring of 6 can be chosen in $\dfrac{7 \times 6 \times 5}{1 \times 2 \times 3}$ or 35 ways, those of the second in $\dfrac{4 \times 3 \times 2}{1 \times 2 \times 3}$, or 4 ways. As the order of the two rings is irrelevant, the product must be divided by 2, making 70 ways. Each ring of 6 containing a given gametic type is susceptible of $2^2 \lfloor 2$ or 8 arrangements. Hence the total number is $70 \times 8^2 \div 2$, or 2240. The total number of types is $\frac{1}{2}(135,135 + 1) \times 135,135$, in accordance with theory.

There is no great difficulty in making a similar calculation for any value of n, but it becomes rather tedious when n is large. However I have tabulated in Table I $r(n)$, the quantity by which $g(n)$ must be

TABLE I.

n	1	2	3	4	5	6	7
$p(n)$	1	2	3	5	7	11	15
$g(n)$	1	3	15	105	945	10,395	135,135
$r(n)$	1	1	4	24	192	1,920	23,040

n	8	9	10	11
$p(n)$	22	30	42	56
$g(n)$	2,027,025	34,459,425	654,729,075	13,749,310,575
$r(n)$	322,560	5,160,960	92,897,280	1,857,945,600

n	12	20	30	40	50
$p(n)$	77	627	5,604	37,338	204,226
$g(n)$	316,234,143,225	$4 \cdot 52 \times 10^{23}$	$4 \cdot 13 \times 10^{40}$	$1 \cdot 12 \times 10^{59}$	$3 \cdot 86 \times 10^{78}$
$r(n)$	40,874,803,200	$3 \cdot 20 \times 10^{22}$	$2 \cdot 38 \times 10^{39}$	$5 \cdot 61 \times 10^{57}$	$1 \cdot 71 \times 10^{77}$

TABLE II.

Pairing type	Number of zygotic types
$7 \times (2)$	$1 \times 135,135$
$(4) + 5 \times (2)$	$21 \times$,,
$2 \times (4) + 3 \times (2)$	$210 \times$,,
$3 \times (4) + (2)$	$420 \times$,,
$(6) + 4 \times (2)$	$140 \times$,,
$(6) + (4) + 2 \times (2)$	$1,680 \times$,,
$(6) + 2 \times (4)$	$1,680 \times$,,
$2 \times (6) + (2)$	$2,240 \times$,,
$(8) + 3 \times (2)$	$840 \times$,,
$(8) + (4) + (2)$	$5,040 \times$,,
$(8) + (6)$	$6,720 \times$,,
$(10) + 2 \times (2)$	$4,032 \times$,,
$(10) + (4)$	$8,064 \times$,,
$(12) + (2)$	$13,440 \times$,,
(14)	$23,040 \times$,,
Total	$67,568 \times 135,135 = 9,130,801,680$

multiplied in order to give the number of zygotic types where all the $2n$ chromosomes are arranged in a ring. $r(n)$ also represents half the number of types with a ring of $2n$ which can produce any one type of gamete. Clearly $r(n) = 2^{n-2} \lfloor n-1$ when n exceeds 1. The fraction of all zygotic types which have a ring of $2n$ chromosomes is $\dfrac{2r(n)}{g(n)+1}$, a fraction which diminishes slowly as n increases. When n is large it approximates (by Stirling's theorem) to $\frac{1}{2}\sqrt{\dfrac{\pi}{n-1}}$, and ultimately vanishes, but for all ordinary values of n the type with all the chromosomes in a ring contributes an appreciable fraction, *e.g.* 26 per cent. when $n = 12$, and 8·9 per cent. when $n = 100$, of all zygotic types.

APPENDIX II.

List of Diploid Forms in *Oenothera* classified according to Configuration and in Order of Frequency with Random Assortment.

1. *Ring of Fourteen:* (14).

[s   *Oe. rosea* (subgenus *Hartmannia*) Schwemmle, 1924.]
s   *Oe. muricata (curvans.rigens)* Cleland, 1923, 1926.
s   *Oe. strigosa (deprimens.stringens)* Oehlkers, 1926.
s   *Oe. Cockerelli (curtans.elongans)* Oehlkers, 1926; Cleland and Oehlkers, 1929.
s   *Oe. sinuata* Sinoto, 1927.
h   *Oe. R-biennis × Hookeri (albicans.*[h]*Hookeri)* Cleland, 1928; Hoeppener and Renner, 1929.
s   *Oe. eriensis* Sheffield, 1927.
[s   *Oe. novae-scotiae* Sheffield, 1927 (=*Oe. muricata*).]
s   *Oe. Agari* Sheffield, 1927.
s   *Oe. pratincola* "C" and "E" $(a+\beta)$ ⎫
m   *Oe. pratincola* mut. *formosa*   ⎬ Kulkarni, 1929 *b*.
s   *Oe. angustissima* Gates and Sheffield, 1929.
h   *Oe. ammophila × novae-scotiae* ⎫
h   *Oe. ammophila × eriensis*   ⎬ Sheffield, 1929.
h   *Oe. grandiflora × biennis cruciata (rubens.truncans)* ⎫
h   *Oe. grandiflora × suaveolens (flavens.truncans)*   ⎬ Gerhard, 1929.
h   *Oe. muricata × grandiflora (rigens.truncans)*
h   *Oe. Cockerelli × grandiflora (curtans.truncans)*   ⎭
s   *Oe. grandiflora* of De Vries *(acuens.truncans)* ⎫
h   *Oe. grandiflora × Hookeri (truncans.*[h]*Hookeri)*
h   *Oe. Lamarckiana × suaveolens sulfurea (albicans.velans)* ⎬ Cleland and Oehlkers, 1929.
h   *Oe. grandiflora × Lamarckiana (acuens.gaudens)*
h   *Oe. Lamarckiana cruciata × strigosa (gaudens.strigens)* ⎭
s   *Oe. pycnocarpa* ⎫
s   *Oe. nutans*   ⎬ Catcheside, 1930.

s   *Oe. pachycarpa (augens . subcurvans)*
h   *Oe. biennis × pachycarpa (albicans . subcurvans)*
h   *Oe. muricata × pachycarpa (rigens . subcurvans)*   } Rudloff, 1930.
h   *Oe. Lamarckiana × pachycarpa (subcurvans . velans)*
[h   *Oe. biennis × Lamarckiana (albicans . velans)* Håkansson, 1930 *a*.]
h   *Oe. suaveolens × Hookeri (albicans .* [h]*Hookeri)* Cleland and Blakeslee, 1930.

2. *Ring of Twelve and One Pair:* (12) + (2).

h   *Oe. franciscana sulfurea (F₂ biennis × franciscana)* Cleland, 1924.

s   *Oe. Lamarckiana (velans . gaudens* and cross-over mutants) Cleland, 1925; Håkansson, 1926; Illick, 1929.

s   *Oe. suaveolens (albicans . flavens)* Oehlkers, 1926; Cleland, 1928; Illick, 1929.

h   *Oe. aurata* (derivative) Cleland, 1928.

h   *Oe. Lamarckiana × biennis* Chicago *(fallax)* Cleland, 1928; Håkansson, 1928.

h   *Oe. biennis × suaveolens* (and reciprocal *rubens . flavens)* Cleland, 1928; Hoeppener and Renner, 1929.

s   *Oe. ammophila (rigens . percurvans)* Sheffield, 1927.

h   *Oe. pratincola* "E" mut. "*formosa*" × *Oe. pratincola* "C" Kulkarni, 1929 *b*; (?) Illick, 1929.

s   *Oe. grandiflora* (of De Vries) Gerhard, 1929. (Cf. Cleland and Oehlkers, 1929.)

h   *Oe. grandiflora × biennis cruciata (albicans . truncans)* Gerhard, 1929.

h   *Oe. eriensis × ammophila*
h   *Oe. eriensis × rubricalyx*   } Sheffield, 1929.
h   *Oe. rubricalyx × novae scotiae*

[m   *Oe.* mut. *pervirens* Illick, 1929.]

h   *Oe. Lamarckiana × suaveolens sulfurea (gaudens . flavens)*
h   *Oe. suaveolens × strigosa* (i) *(albicans . stringens)*
                         (ii) *(deprimens . flavens)*   } Cleland and Oehlkers, 1929.
h   *Oe. suaveolens × Cockerelli* (i) *(albicans . elongans)*
                         (ii) *(curtans . flavens)*

h   *Oe. (purpurata × Lamarckiana) × purpurata (*[h]*purpurata . velans)* Rudloff, 1929.

h   *Oe. pachycarpa × Lamarckiana (augens . velans)* Rudloff, 1930.

3. *Rings of Ten and Four:* (10) + (4).

h   *Oe. aurata* (derivative) Cleland, 1927.

h   *Oe. grandiflora × Lamarckiana* (i) *(truncans . velans)*   } Cleland and Oehlkers, 1929.
                                 (ii) *(truncans . gaudens)*   } (Cf. Gerhard, 1929.)

h   *Oe. grandiflora × biennis cruciata (rubens . acuens)* Gerhard, 1929.

h   *Oe. grandiflora × cruciata (truncans . flectens)* Gerhard, 1929.

4. *Rings of Eight and Six:* (8) + (6).

s   *Oe. biennis (rubens . albicans)* Cleland 1923, 1926 *a*; Emerson, 1924; Kihara, 1927.

s   *Oe. rubricalyx (*[rb]*rubens . tingens)* Rudloff, 1929.

h   *e. biennis × Lamarckiana (albicans .* (?) *gaudens)* Håkansson, 1930 *a*.

h   *Oe. Lamarckiana × suaveolens sulfurea (albicans . gaudens)* Cleland and Oehlkers, 1929.

5. *Rings of Eight and Four, and One Pair:* (8) + (4) + (2).

h   *Oe. muricata × grandiflora (rigens . acuens)* Gerhard, 1929.

6. *Ring of Ten and Two Pairs:* $(10) + 2\,(2)$.

h   *Oe. biennis* × *Hookeri* (*rubens*.^{h}*Hookeri*) Cleland, 1928.

h   *Oe. Hookeri* × *Lamarckiana* (^{h}*Hookeri*.*gaudens*) Hoeppener and Renner, 1929.

h   *Oe. Lamarckiana cruciata* × *strigosa* (i) (*deprimens*.*velans*) } Cleland and Oehlkers,
                                        (ii) (*deprimens*.*gaudens*)}   1929.

h   *Oe. Lamarckiana* × *grandiflora* (*acuens*.*gaudens*)

h   *Oe. grandiflora* × *biennis cruciata* (*albicans*.*acuens*) } Gerhard, 1929.

h   *Oe. grandiflora* × *muricata* (*curvans*.*truncans*)

h   *Oe. pachycarpa* × *Hookeri* (^{h}*Hookeri*.*augens*) Rudloff, 1930.

h   *Oe. Hookeri* × *biennis* (^{h}*Hookeri*.*rubens*) Cleland and Blakeslee, 1930.

h   *Oe. purpurata* × *Lamarckiana* F_2 (^{h}*purpurata*.*gaudens*) Rudloff, 1929.

h   *Oe.* (*purpurata* × *suaveolens*) × *biennis* (*rubens*.*flavens*) Rudloff, 1929.

7. *Two Rings of Six and One Pair:* $2\,(6) + (2)$.

None.

8. *Ring of Six and Two Rings of Four:* $(6) + 2\,(4)$.

h   *Oe. suaveolens* × *pachycarpa* (*flavens*.*subcurvans*) F_1 Rudloff, 1930.

9. *Rings of Six and Four and Two Pairs:* $(6) + (4) + 2\,(2)$.

h   *Oe. biennis* × *muricata* (*albicans*.*curvans*) Cleland, 1928.

hd *Oe. suaveolens* × *pachycarpa* F_2 (partly a-lethal derivative (i)) Rudloff, 1930.

h   *Oe. grandiflora* × *Lamarckiana* (*acuens*.*velans*) Gerhard, 1929; Cleland and Oehlkers,
        1929.

h   *Oe. Lamarckiana cruciata* × *strigosa* (*velans*.*stringens*) Cleland and Oehlkers, 1929.

10. *Ring of Eight and Three Pairs:* $(8) + 3\,(2)$.

m   *Oe. rubricalyx* Cleland, 1929 (? *rubricalyx* × *grandiflora* derivative) Gates and Sheffield,
        1929; Emerson, 1931 (=modified *velans*.^{h}*latifrons*).

hd *Oe. ammophila* × (*biennis* × *rubricalyx*) F_2 Gates and Sheffield, 1929.

m   *Oe. simplex elongata* (segregate of *simplex* ex $2n+1$ *oblonga*)

m   *Oe. distans* (? given by Boedijn as $2n+1$) (ex $2n+1$ *nitens*) } Håkansson, 1930 *a*.
                        (cf. De Vries, 1923)

h   *Oe. suaveolens* × *Cockerelli* (*flavens*.*elongans*) Cleland and Oehlkers, 1929.

m   *Oe. grandiflora* × *Hookeri*, mutant, Gerhard, 1929.

11. *Three Rings of Four and One Pair:* $3\,(4) + (2)$.

None.

12. *Two Rings of Four and Three Pairs:* $2\,(4) + 3\,(2)$.

h   *Oe. Hookeri* × *Lamarckiana* (^{h}*Hookeri*.*velans*) Hoeppener and Renner, 1929.

h   *Oe. Lamarckiana* × *suaveolens sulfurea* (*velans*.*flavens*) Cleland and Oehlkers, 1929.

h   *Oe. chicagoensis* × *suaveolens* (*excellens*.*flavens*) Cleland and Blakeslee, 1930.

13. *Ring of Six and Four Pairs:* $(6) + 4\,(2)$.

m   *Oe. rubrinervis* (*paenevelans*.*subvelans* ex *Lamarckiana*) Cleland, 1925; Illick, 1929.
                        =*rubrisepala* Håkansson, 1926.

[m *Oe. erythrina* (=*rubrinervis*?)

m   *Oe.* "mut. *sulfurea*"

h   *Oe. aurata* × *latifrons*   } Cleland, 1928.

h   *Oe. franciscana sulfurea* × *latifrons*

h   *Oe. grandiflora* × "mut. *sulfurea*" [and reciprocal]

hd *Oe. ammophila × rubricalyx* (derivative) Sheffield, 1929.

hd *Oe. suaveolens × pachycarpa* F_2 (partly a-lethal derivative (ii)) Rudloff, 1930.

h   *Oe. seg. decipiens × grandiflora* [and reciprocal] Illick, 1929.

h   *Oe. Lamarckiana × chicagoensis* (*velans . excellens*) Cleland and Blakeslee, 1930.

14. *Ring of Four and Five Pairs:* (4) + 5 (2).

s   *Oe. franciscana* Cleland, 1922; Hoeppener and Renner, 1929.

h   *Oe. Hookeri × suaveolens* (*flavens .* [h]*Hookeri*) Cleland, 1928; Cleland and Blakeslee, 1930.

h   *Oe. biennis × muricata* Cleland, 1928.

h   *Oe. grandiflora × franciscana* [and reciprocal] Cleland, 1928.

h   *Oe. aurata × latifrons* Cleland, 1928.

m   *Oe. rubrisepala a* (ex *Lamarckiana*) Håkansson, 1930 *a*.

h   *Oe. suaveolens sulfurea × strigosa* (*flavens . stringens*) Cleland and Oehlkers, 1929.

h   *Oe. chicagoensis × Hookeri* (*excellens .* [h]*Hookeri*) Cleland and Blakeslee, 1930.

h   *Oe. purpurata × cruciata* ([h]*purpurata . flectens*) Rudloff, 1929.

h   *Oe. grandiflora × suaveolens* (*flavens . acuens*) ⎫
h   *Oe. Lamarckiana × grandiflora* F_2 (*acuens . velans*) ⎬ Gerhard, 1929.

15. *Seven Pairs:* 7 (2).

s   *Oe. grandiflora* Ait. Davis, 1909; Cleland, 1928; Illick, 1929.

s   *Oe. Hookeri* ([h]*Hookeri .* [h]*Hookeri*) Schwemmle, 1924; Hoeppener and Renner, 1929.

m   *Oe. blandina* (ex *Lamarckiana*) Cleland, 1925.

m   *Oe. deserens* (*subvelans . subvelans* ex *rubrinervis*) Cleland, 1925; Håkansson, 1930 *a*.

h   *Oe. suaveolens × strigosa* (*flavens . stringens*) Oehlkers, 1926.

m   *Oe. latifrons* (ex *rubricalyx*) Cleland, 1928.

h   *Oe. grandiflora × mut. sulfurea* [and reciprocal] Cleland, 1928.

hd *Oe. Lamarckiana × grandiflora* (*truncans . velans*) Gerhard, 1929.

hd *Oe.* (*biennis × rubricalyx*) *× ammophila* F_5 Gates and Sheffield, 1929 *b*.

s   *Oe. franciscana* Kulkarni, 1929 *a*.

hd *Oe. lutescens* (*flavens . flavens* ex *suaveolens × biennis*) ⎫
m   *Oe. fragilis* ⎬ Hoeppener and Renner, 1929.

hd *Oe. grandiflora × seg. decipiens* [and reciprocal] Illick, 1929.

m   *Oe. mut. pervirens* Illick, 1929.

m   *Oe. grandiflora mut. ochracea* (*acuens . acuens*) Gerhard, 1929.

s   *Oe. purpurata* Rudloff, 1929.

h   *Oe. purpurata × suaveolens* F_2 ([h]*purpurata . flavens*) Rudloff, 1929.

Note. s, species; m, mutant; h, hybrid; hd, hybrid derivative. Forms, for various reasons, not included in summary (Section XII) in parenthesis. Mutants that are probably equivalent to species in pairing properties are included with the species. Hybrid derivatives supposed to be the same as the F_1 are included as "hybrids."

An earlier list has been compiled by Gerhard (1929) and a summary by Sheffield (1929), but they have counted identical types with different names separately.

APPENDIX III.

Telosynapsis and Interpretation.

Telosynapsis was the notion that the chromosomes united end to end in a "continuous spireme" at the prophase of meiosis, and that pairing at the metaphase was the result of the "segmentation" of this

spireme, *i.e.* its breakage at alternate unions. Ring-formation was then the result of the (unexplained) failure of this supposed segmentation.

The doctrine was based on (i) faulty interpretation and (ii) faulty reasoning. The interpretation (of Haecker) was at fault because it related the double (bivalent) pachytene thread to the double (univalent) prophase thread of mitosis. The reasoning which maintained it in its later years was at fault because it argued from the premise that chromosomes were permanent units of inheritance, whereas the species whose ring formation appeared to lend weight to the telosynaptic point of view were precisely those in which the chromosomes had not behaved as units. The permanence of the chromosome as a unit was disproved by McClung in 1917, yet in 1923 (Gates) the application of the theory of telosynapsis to *Oenothera* was defended on the ground that no other theory was compatible with the doctrine of permanence.

This led to a curious fallacy. The conception of homology rested on the discovery that *chromosomes* were different (*a*) morphologically and (*b*) physiologically. Chromosome pairs were seen to correspond morphologically, and were inferred to correspond physiologically, from the fact that they *segregated at random* with regard to one another. On the theory of telosynapsis the structure of the chromosomes was supposed to be the same with or without ring formation: the chromosomes were genetically pairs of units *although pairs did not segregate at random*. This view involved two contradictory assumptions. Pairing at one union was supposed to be due to homology, at the next to non-homology. In this way one could make the ring of fourteen chromosomes in *Oenothera* by contradicting oneself thirteen times.

The result was that the valuable idea of homology lost all significance in discussions of *Oenothera*. For example we may take the relationship of the theory of chromosome pairing with homology of chromosome structure. The following table shows that every possible combination of assumptions has been made explicitly or implicitly.

In some cases (Schwemmle, 1926; Kihara, 1927) assumptions have been made on the basis of analogy, and logically applied. In other cases this is not so. The logical confusion is illustrated by Gates' arguments (1930). He concludes that the *assumption* of segmentally interchanged constitution may follow as well from the *assumption* of telosynapsis as of parasynapsis, because (he believes) the *fact* of interchange might just as well follow the *fact* of telosynapsis as the *fact* of parasynapsis. But if we believe in telosynapsis there is no ground for supposing that segmental interchange has taken place at all, in *Oenothera, Datura* or in any other

organism; for segmental interchange is inferred on the assumption of specific pairing properties which are denied by the theory of telosynapsis. This is an example of the results of the random selection of hypotheses.

Assumptions	Telosynapsis	Indefinite	Parasynapsis
Homologous and non-homologous elements pair alternately	Gates (1907–1929) Cleland (1922–1929) Håkansson (1927) Sheffield (1929) Kulkarni (1929)	Gates (1928) Cleland (1929)	Boedijn (1924) Schwemmle (1926) Kihara (1927)
Only homologous elements pair	Sheffield (1929, p. 220) Håkansson (1928) Cleland and Blakes- lee (1930) Gates (?1930)	Håkansson (1930) Capinpin (1930)	Darlington (1929) Catcheside (1931)

In view of such fallacies it is unfortunate that Cleland and Blakeslee (1930) have also failed to distinguish between the two hypotheses of specificity and interchange. They have applied to *Oenothera* the hypothesis that the pairing properties of the chromosomes are specific to their parts. This hypothesis, as I have pointed out (1929 a), almost certainly requires the further assumption that an exchange of segments has occurred at some time in the history of the race between non-homologous chromosomes in non-ring-forming individuals. But a test of the hypothesis of *specificity* has no relation to a test of the hypothesis of *interchange*. (It is these two hypotheses that Gates has confused.) Yet Cleland and Blakeslee refer to their tests in every case as tests of the "hypothesis of interchange," when they are actually testing the hypothesis of specificity. The two types of tests, the first of which I described in 1929 and the second of which I have described above (Sections VI and X), have no necessary relationship.

The logical absurdity of the telosynaptic system (which has been exposed from various points of view by Whiting (1923), Renner (1929), and myself (1929 b and c)) was thought to be compensated by the soundness of the interpretation of the prophase of meiosis. But it is perhaps appropriate to enquire what is the basis of good interpretation in cytology of the kind we are considering.

Good interpretation is relative and depends on the degree of understanding in physiological terms of the structures observed morphologically (*i.e.* not by inference) in good fixation. The *primary* criterion of good fixation is the extent to which it reveals structures that can be related to those observed (i) at different stages in the same species, or

(ii) at the same stage in different species, or (iii) at the same stage in living material in any species. A *secondary* criterion of good fixation in the prophase of meiosis—derived from applying the general criterion in plants and animals—is the texture of chromosomes after fixation and staining, which should be granular up to a late stage, and not homogeneous and smooth surfaced as in all prophase observations of *Oenothera* and other supposedly telosynaptic organisms. Structures observed in the prophase of *Oenothera* and other genera, and described as evidence of telosynapsis by Gates, Davis, Latter, Sheffield, Cleland, Kulkarni and Håkansson, were unintelligible physiologically and failed to satisfy any of these primary criteria. On secondary criteria such as the texture of the prophase chromosomes they were equally at fault. For these reasons I have ignored them in considering the theory of ring formation (1929 *a*) and confined myself to the observations that did fulfil these conditions, viz. the observations of diakinesis and metaphase. To understand the prophase stages of which I considered we had no direct knowledge I have turned to analogy with other organisms on which observations fulfilled the required conditions. In this way it seems possible to arrive at a good interpretation by an indirect method. The criterion of its goodness is then the degree to which its physiological implications can be verified.

The present study is an examination of this problem. It is a consideration of hypotheses that relate the genetical and cytological observations in *Oenothera*. Before it can be considered, the conclusions that are held to follow directly from observation must first be studied. They have been most concisely described by Sturtevant (1925) in dealing with the genetical work of Renner, and by Cleland and Oehlkers (1929) in dealing with their cytological work.

<div style="text-align: center">APPENDIX IV.</div>

<div style="text-align: center">DEMONSTRATIONS OF CROSSING-OVER.</div>

There are three conceivable ways of proving cytologically that crossing-over has preceded the formation of a chiasma:

1. In a structural hybrid, with the chromosomes homologous but dissimilar at both ends, observation of a single interstitial chiasma would distinguish between crossing-over and failure of crossing-over. This method of demonstration has been sought for but not yet found.

2. In a tetraploid certain quadrivalent configurations distinguish the occurrence of crossing-over similarly. This has been shown in *Hyacinthus* (Darlington, 1930 *c*).

3. In a ring-forming structural hybrid a chiasma between interstitial parts of two chromosomes whose ends are not homologous would distinguish the occurrence of crossing-over. This test is equivalent to the first; the difference is that it depends not on morphological but on physiological distinctions. It depends on the assumption that the pairing properties of chromosome parts are specific.. This has been amply demonstrated in *Oenothera* by all recent cytological studies. Alternative hypotheses are considered elsewhere (Darlington, 1929 *b* and *c*, 1931 *a*, *b*, *c*).

APPENDIX V.

The theory of the origin of complexes described above depends on one important assumption, viz. that the amount of pachytene pairing of two corresponding short segments of chromosome is diminished when their homology is not continued in adjoining segments. Hence translocations taking place at random in parts of two homologous sets of chromosomes will eventually reduce the amount of pairing between those parts (*e.g.* in the differential segments of the complex heterozygote) to a negligible quantity.

This hypothesis has seemed to me to be plausible because it can be seen that the chromosomes pair in a linear sequence and where this sequence is interrupted a delay in finding a new sequence is inevitable, since the chromosomes before pairing are distributed at random in the nucleus (Darlington, 1931 *b*). It is not necessary therefore to assume any special properties such as rigidity in the chromosome thread at zygotene.

The hypothesis has been borne out by observations of "differential affinity" in the chromosomes of polyploids and their hybrids (cf. Darlington, 1931 *b*).

I have therefore suggested that long chromosomes (1930 *c*) and chromosomes with a continuous linear homology (see above) have an advantage in pairing over those containing shorter homologous segments.

These views are supported by Dobzhansky's observations (*Amer. Nat.* 65, pp. 214–32, received since going to press) of the reduction of crossing-over in *Drosophila* heterozygous for translocations (*i.e.* structurally hybrid), although his assumption that rigidity is the cause seems unnecessary. Dobzhansky assumes that this reduction depends on a reduction in the length of chromosome paired at pachytene and *not* on a reduction of crossing-over in the unit of length paired. This is

the same as my assumption that the differential segments in *Oenothera* fail to cross over because they fail to pair, but in both cases the alternative remains a possibility (cf. Section VIII (2)). The result however is indifferent: in the region where the homology changes the frequency of crossing-over is reduced. Genetic evidence therefore agrees with cytological evidence. in suggesting that translocation sufficiently explains the suppression of crossing-over between complexes in *Oenothera.*

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EXPLANATION OF PLATE XII.

Microphotographs taken by Mr H. C. Österstock with his own cinema camera; showing the same object at different focuses.

Figs. 1–6. Metaphase of mitosis from the root-tip. × ca. 1500.

Figs. 1 and 2. *Oe. berteriana*, $2n = 14$. Cf. constrictions and trabants in fig. 2 with those shown in drawing of Text-fig. 1.

Figs. 3, 4 and 5. *Oe. Lamarckiana × Oe. albiflexa gigas*, $2n = 22$. Cf. Text-fig. 4.

Fig. 6. *Oe. albiflexa gigas*, $2n = 28$. Cf. Text-fig. 2.

Figs. 7–19. Side views of metaphase and anaphase of first pollen mother-cell division. Showing different types of configuration and separation of bivalents, resulting from different types of chiasma formation.

Figs. 7 and 8. Regular disjunction of part of ring with only terminal chiasmata in *Oe. biennis*, A. *Note*: the ends of some of the chromosomes which are square instead of pointed in the photograph are those in which the two chromatids are seen sideways instead of endways. × ca. 6000.

Figs. 9 and 10. Bivalent with interstitial chiasma (cut cell) also illustrated in Text-fig. 16 A. Both distal arms are lying in the equatorial plane but one is oblique to the line of vision. × ca. 4000.

Fig. 11. Bivalent with interstitial chiasma and the distal arms attached by terminal chiasmata with chromosomes passing to opposite poles. The distal arms are therefore lying in the axis of the spindle. × ca. 2400.

Figs. 12–16. Same type of chiasma formation (giving the figure-of-eight or branched ring) as in fig. 11. Illustrated also in Text-fig. 18. *Note*: (i) the arms distal to the interstitial chiasma are pulled into the axis of the spindle by their terminal attachment to two other chromosomes; (ii) the pair to the right show the characteristic form of chromosomes attached at one end (terminally) but not at the other, *i.e.* making a rod not a ring. Figs. 12 and 16, × ca. 2000; figs. 13, 14, 15, × ca. 2400.

Figs. 17–19. Anaphase: pair of chromosomes lagging owing to the greater resistance to separation of the chromatids where the chiasma is interstitial. Illustrated also in

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